THE ANATOMY OF THE TREMATODE, *MACROPHYLLIDA ANTARCTICA* (HUGHES)

by

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In 1928 Miss W. K. Hughes described amongst other heterocotylean trematodes from fish, a new genus and species, *Macrophylla antarctica*, from the gills of a shark, *Mustelus antarcticus*, from Port Phillip, Victoria, and mentioned that the species was met with on two specimens out of about one hundred examined. As the generic name was preoccupied, *Macrophyllida* was proposed in its place by Johnston (4). Thanks to the kindness of Dr. O. W. Tiegs, an opportunity was afforded to re-examine the material, all of which is mounted, so that a more detailed and, to some extent, emended account may be given.

The largest specimen measures 14·8 mm. long, with a maximum breadth of 2·8 mm.; excluding the posterior sucker, the length is 14 mm. The anterior end is broadly rounded and separated from the body by a well-marked constriction on either side. The body has a fairly uniform breadth in its anterior two-thirds, and then tapers gradually to a rounded extremity bearing the disc or sucker, which is nearly circular, with a diameter of 2mm.

The anterior end bears a pair of extensive glandular areas which almost meet in front, but whose posterior borders trend outwards and backwards to terminate just in front of the notch on the corresponding side of the body. Each area is traversed ventrally by rather low, flattened ridges, which vary in length according to their positions, the largest being nearly a millimeter. The main ridges tend to coalesce as they approach the inner margin of the area, while their outer extremities are separated by smaller ridges, so that the grooves between the main ridges commonly bifurcate to enclose the smaller partially. They are short and rather indistinct at the anterior end of the worm, where they are directed forwards. Elsewhere they are directed outwards. There are probably between fifty and sixty on each side, about half of them extending from the inner to the outer edge of the area. The ridges stain deeply in whole mounts as well as in sections on account of the presence of abundant, very narrow ducts, which open on them. These ducts pass upwards and inwards, except in the inner parts of the ridges where they travel inwards and slightly outwards, towards certain cells

which occupy most of the upper half of transverse sections of the region. These small pyriform cells, which are unicellular glands, possess a relatively large nucleus and poorly staining cytoplasm. The ventral surface between the two glandular areas is flat and more or less triangular.

The disc, or posterior sucker, has a thin membranous outer area with weak radiating and circular muscular fibres, but the remainder of the organ is well supplied with powerful dorsoventral, oblique and circular muscles. It is traversed by five muscular septa representing the anterior, two anterolateral, and two posterolateral radii of *Capsala (Tristoma* of authors*)*. The posterior loculus is large and is crossed by two obsolescent radii devoid of special musculature, but provided each with two delicate flexible chitinous rodlike hooks, the anterior measuring 0·095 mm. and the posterior 0·05 mm. long. The anterior end of the former and the posterior end of the latter are pointed, while the opposite extremities are slightly broadened. Strong tranverse fibres pass across the disc, along the transversely placed, i.e., the lateral, septa. In the tissue between the two hooks is a large cell with processes and with a large circular nucleus, probably a multipolar nerve cell. Two similar cells are to be seen on most of the well-developed radii, one near the central loculus and the other nearer the margin of the disc, while others are present near the centre of the disc.

Below the cuticle is a fairly wide subcuticula, succeeded by a well-defined, but narrow, zone of circular muscle fibres, arranged as two layers, closer dorsally than ventrally. Within this are very abundant, delicate, diagonal muscles, and below these are the longitudinal fibres, which are fewer and larger. The dorsoventral fibres are numerous and well-developed. In some transverse sections the ventral region shows a well-marked third circular layer, but it is probably a locally modified portion of the diagonal system which appears to be absent in such positions. In the vicinity of the pharynx the muscles are more pronounced, and the diagonal series tends to assume a transverse position.

The mouth is a large crescentic aperture a little in front of the line joining the lateral notches, and may partly underlie the posterior pair of eyes. Projecting into the buccal cavity is the pharynx, composed chiefly of large gland cells resembling those described by Heath (2) as occurring in *Epibdella squamula*.

EXPLANATION.

Fig. 1. Entire worm, from the dorsal aspect. Vitellaria and greater part of digestive system omitted. Vitelline field dotted. Drawn from type slide to scale indicated.

Fig. 2. Disc.

Fig. 3. Female reproductive system and male duct.

Fig. 4. Arrangement of muscle fibres in vicinity of genital apertures when the latter are closely approximated. Cirrus partly protruded.

References to lettering: cs, cirrus sac; gr, glandular ridges (grooves dotted); i, intestine; l, vaginal lips; ma, male aperture; od oviduct; ot, ootyp; ov, ovary; pl, posterior loculus; pr, prostate duct; sal, salivary glands; sg, shell glands; t, testis; ua, uterine aperture; v, vagina; vd, vas deferens; vs, vesicula seminalis; vt, vitellaria; vtd, vitelline duct; vtr, vitelline reservoir.

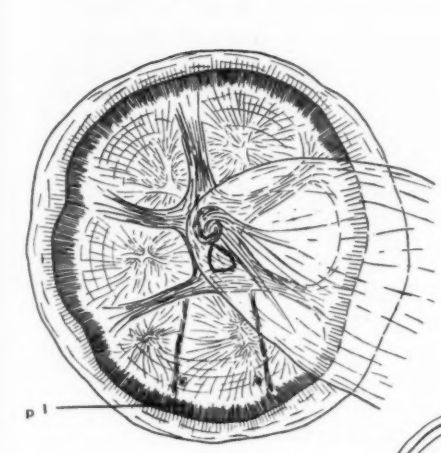

Fig. 2

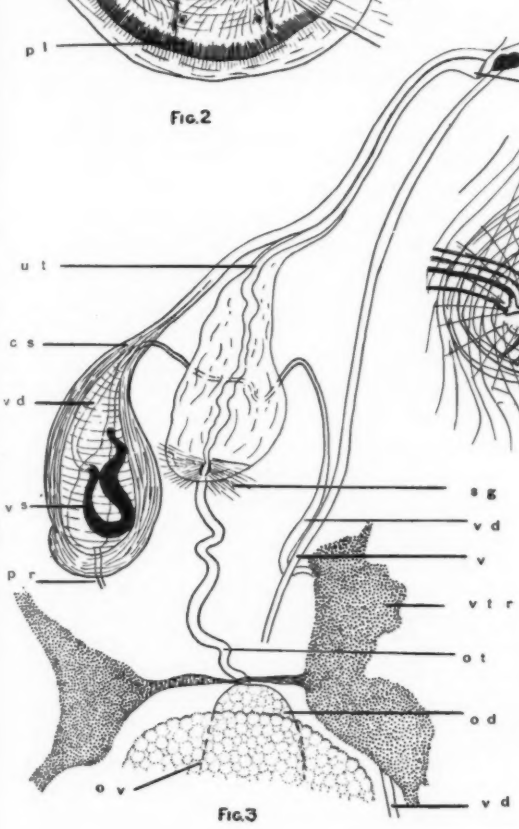

Fig. 3

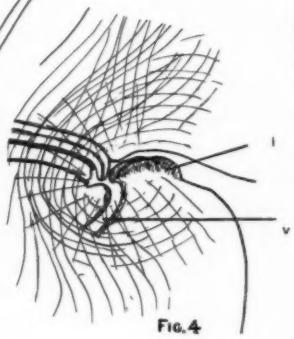

Fig. 4

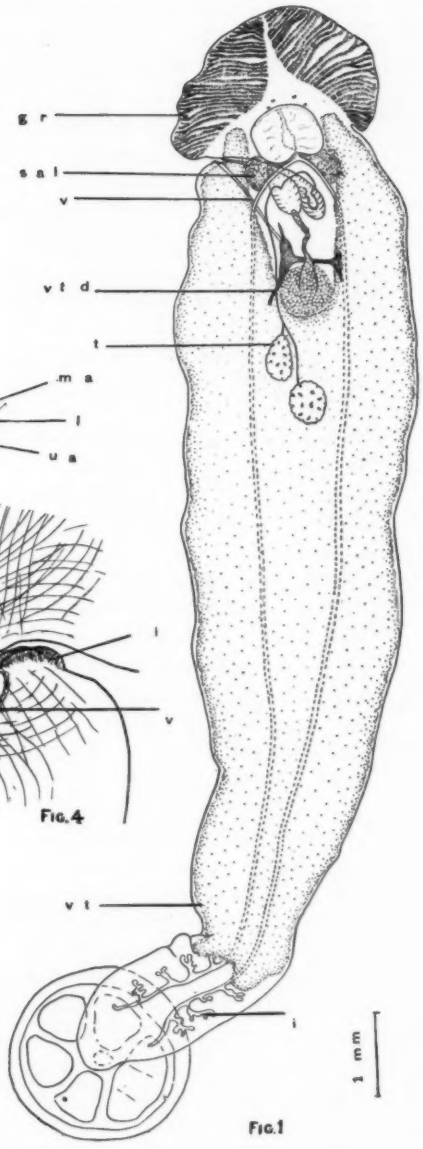

Fig. 1

They have a large nucleus in the basal region of the cell; the contents (probably a secretion) are finely granular and deeply staining. The free extremity projects into the lumen of the organ. The pharynx is nearly circular in most specimens and measures 0·85 mm. across by 0·7 mm. long, in the type specimen. In other cases it is relatively larger and narrower, and its anterior region is more elongated and projects forwards into the buccal cavity. The organ extends almost from the dorsal to the ventral surface. Surrounding it are well-developed circular muscle fibres, while its inner region contains numerous longitudinal and circular fibres close to the lumen. Radiating fibres are strongly developed.

Into the short thick-walled oesophagus there enter a great number of very delicate salivary ducts with deeply staining contents. These are very long, and extend outwards to their respective cells which form a dense mass occupying a triangular area on either side between the posterior edges of the pharynx, the vitelline field, and the cirrus sac or uterus respectively. They more or less overlie the intestinal crura and extend outwardly to the region of the inner longitudinal nerve. The ducts converge towards the oesophagus to form a compact mass of fine tubes.

The intestinal crura are long, narrow, and more or less straight, extending almost to the end of the worm. There are no caeca on the inner aspect, but on the outer a number of short branching caeca are given off at intervals. As the whole of the region, except its most posterior portion, is abundantly supplied with vitelline vesicles, the arrangement of these caeca could not be determined, though Miss Hughes has indicated their position in her figure. Their arrangement at the end of the parasite is, however, figured in this account. The main crura lie just laterally from the ovary, cirrus sac and uterus, but above and just inwardly from the inner longitudinal nerves and the excretory canals, the latter lying between the corresponding crus and nerve. The intestinal cells are flattened and project slightly into the lumen. Their small nuclei have a deeply staining karyosome and are near the base of the cells. A branch from each crus arises anteriorly and passes forwards laterally to the pharynx, the two converging in front of the anterior nerve commissure to fuse in the midline and extend forwards for a short distance.

The brain lies just in front of the pharynx and occupies nearly two-thirds of the thickness of the section. It is traversed by many dorsoventral muscle fibres. It has large nerve cells in its dorsal and lateral regions, near the anterior pair of eyes. The nerve fibres are mainly longitudinal in the middle of the brain, but transverse dorsally and ventrally, especially ventrally. In the posterior part of the organ the fibres are chiefly transverse, and eventually pass outwards and backwards as the main longitudinal nerves, the larger inner trunk being close to the pharyngeal wall, while the outer comes to occupy a more lateral position. These nerves are connected at irregular intervals in the body by commissures. From each side of the anterior part of the brain a nerve is given off to the pos-

terior part of the glandular area, and another large nerve passes forwards just inwardly from each area, to become connected with its fellow of the opposite side by a wide commissure which has nerve cells, some of them tripolar. These anterior longitudinal nerves travel further forwards and become joined by another commissure in front of which there are four main nerves distributed to the anterior glandular region, the larger pair being closer to the mid-line. All of them are near the ventral surface. The nervous system of the posterior sucker was not accurately traced, but there is a large branching nerve supplying each loculus. The prominent nerve cells on the radii have been mentioned already.

The eyes are arranged as in *Epibdella* and *Tristoma* (Heath, Goto), those of the anterior pair being closer than those of the posterior. They have a similar structure, each possessing a single layer of small rounded pigment granules on its upper surface, covering a relatively large lens, associated with which is a nerve cell.

The excretory system has not been traced in detail. The main canal of each side travels posteriorly in company with the inner longitudinal nerve, which it overlies. It courses above the vagina for a considerable distance. As a part of these canals becomes much widened and extends dorsally beyond the upper vitelline follicles in the vicinity of the sex apertures and the duct appears as a narrow tube anteriorly to this region, it is probable that the excretory pores are situated close by, though they were not detected in sections. The anterior canals join dorsally in front of the pharynx and above the brain, and from this vessel others arise and pass forwards.

There are two testes, the left being a short distance in front of the right. They measure about 0·6 mm. long by 0·45 mm. in width, though in the type specimen the anterior is rather narrower (0·36 mm.). They are almost circular in section. Each is perforated by feebly staining tissue so that the organ does not appear solid. The vas efferens arises near the anterior border of the posterior testis and travels forwards near the mesial border of the left testis, in front of which it receives the duct from that gland. The vas deferens now proceeds anteriorly just to the left of the ovary, and above the left vitelline reservoir, eventually becoming bent inwards and then forwards again. It then crosses above the uterus and the narrow anterior part of the cirrus sac and travels back close beside the wall of the latter, eventually entering it laterally almost at right angles. Within the sac it becomes somewhat swollen and passes to the inner end of that organ where it forms a loop and then is directed forwards, entering the ejaculatory duct some distance in front of the point of entry of the vas into the sac. The vas deferens has longitudinal and circular muscle fibres in its wall near its point of entry into the sac. The latter is surrounded by a thick mass of muscle fibres, especially longitudinal and oblique, while within these is a well-marked layer of circular fibres. Surrounding the sac, as well as within it, is a loose connective tissue. Several prostate ducts enter the sac near its posterior end and discharge

into a small prostate reservoir. The latter becomes narrowed and joins the vas to form the ejaculatory duct which is rather narrow and muscular. In some specimens a short cirrus was seen protruding above the uterine aperture and directed towards the vaginal orifice, actually entering it in one case. The outer part of the sac is relatively long and tubular and lies immediately in front of, and just dorsally from, the uterus.

Occupying most of the region between the ovary, vitelline fields, uterus and cirrus sac are masses of irregular or pyriform cells, some of them shell glands, while others, which are larger, are probably prostate, and cells similar to them are abundant, more particularly in a ventral situation, even behind the testes. They are finely granular, with large, deeply staining granules in their cytoplasm.

The ovary is a rounded organ about 0·6 mm. in diameter lying in a more or less median position in front of the testes, and a short distance behind the pharynx. The oviduct is a very wide tube arising centrally, and surrounded by connective tissue, which increases in amount as the duct passes upwards and forwards within the capsule of the gland. The duct diminishes in size until, on leaving the ovary, it is a narrow tube with longitudinal and circular muscle fibres. It is joined by the vitelline duct close to the anterior edge of the ovary, and then travels forwards, becoming somewhat twisted, finally entering the uterus dorsally in its posterior region. Just before doing so it receives a large number of minute, but very long, ducts from the shell glands lying in the vicinity.

The uterine cavity when devoid of an egg collapses, so that the organ, seen in section, appears to be filled with a very loose connective tissue in which a few cells are to be seen. Muscle fibres seem to be wanting. The uterus, regarded as vagina by Miss Hughes, lies closely against the anterior part of the cirrus sac. It diminishes rapidly in size as it passes below the left intestinal crus, and travels in company with, and immediately behind, the narrow outer tubular portion of the sac, both ducts curving outwards and opening below the longitudinal nerves, which become displaced dorsally in this situation. The uterine aperture to the exterior is rather wide, and lies just behind the male opening and close to the vaginal aperture which is placed laterally from it. There is a single oval egg. The only one observed was much distorted, but Miss Hughes gives the following dimensions—about 220 μ by 107 μ.

The three sex apertures are lodged near the bottom of the notch on the left side of the animal at about the level of the posterior margin of the pharynx. The musculature in the vicinity is specially modified so as to become arranged as radiating and more or less circular fibres, so that the notch may serve as a genital sucker, the cavity becoming largely obliterated at times by muscular action and thus causing the male and vaginal apertures to become closely approximated. In one case the end of the cirrus was seen to have entered the vagina. The opening of the latter canal faces forwards and somewhat downwards, and is guarded by

two small lips of a chitinoid material, these lips lying above, and outwardly from, the entrance.

The vagina curves backwards and inwards, at first laterally to the excretory canal, but later below it and both longitudinal nerves as well as the intestinal crus and yolk duct of the same side. It then travels near the uterus, extending beyond it and curving to lie near the vitelline reservoir. Its junction with the rest of the female system was not detected in the defective series of sections examined, but probably unites with the reservoir or the common vitelline duct just in front of the ovary. It has a row of strong longitudinal fibres outside some delicate circular fibres which are adjacent to the lumen. The tube is surrounded by loose connective tissue.

The yolk follicles are very abundant and occupy a field extending from the sides of the pharynx, almost to the posterior end of the worm, excluding a small region between the pharynx and ovary. They are arranged dorsally and ventrally and occur between the testes and ovary and may partly overlap the former. They are elliptical, measuring 0·02 to 0·06 mm. long by 0·02 to 0·04 mm. wide. The secretion is collected by lateral ducts and emptied into the longitudinal duct of each side, which travels in the vicinity of the intestine. Immediately in front of the ovary each forms a yolk reservoir, the two becoming connected by a delicate transverse vitelline duct which lies dorsally to the anterior edge of the ovary and joins the oviduct. The left reservoir is much larger than its fellow in all the preparations examined.

The general anatomy of the parasite indicates that it belongs to the Capsalidae. Its organization is nearer to that of *Capsala (Tristoma* of authors*)* than that of the other genera, but it differs in several striking features which are regarded as being of generic value, such as the presence of two testes which are not in the same plane; the absence of anterior suckers; the characteristic fan-like arrangement of the anterior glandular areas; the contiguity of the three sex apertures marginally; the much simpler intestine devoid of inner caeca; and the virtual suppression of the two posterior radii of the disc. The genus may be placed provisionally in the sub-family Capsalinae.

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A NEW SPECIES OF TREMATODE, OF THE GENUS ANOPLODISCUS

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ANOPLODISCUS AUSTRALIS n.sp.

Plate Figs. 1–3.

The material examined consists of a single mounted preparation of a worm collected by the late Professor S. J. Johnston from the fin of a black bream, *Sparus australis*, from Sydney Harbour. Unfortunately the specimen was compressed unduly, so that some organs have become misplaced or distorted. In view of the fact that the parasite belongs to a genus not hitherto recorded from Australia, an account is now given, but it is hoped that further material may become available in the future, so that an amended and amplified description may be supplied.

The worm, as mounted, measures 2·8 mm. long by 1·4 mm. in maximum breadth, but the real dimensions would probably be about 2·5 mm. by 1 mm. The general shape is fusiform, with broadly truncate ends, the anterior being somewhat rounded and lobed, while the posterior bears the prominent disc, whose diameter (0·75 mm.) is about the same as that of the adjacent portion of the body, the real measurement being probably about 0·6 mm.

The anterior extremity bears two low lobes. The region laterally from, and anterior to, the pharynx and brain contains abundant, relatively large, gland cells, but their ducts are not obvious. There is a shallow area ventrally on each of the lobes, which probably represents the glandular depression referred to by Monticelli in his brief account of *Anoplodiscus richiardii*. The disc is terminal, unarmed, and almost circular. Its musculature consists of very delicate radial and circular fibres.

The crescentic mouth leads into a pharynx, which appears almost circular, perhaps due to compression. The very narrow oesophagus receives a great number of fine ducts from the salivary glands lying laterally between the anterior boundary of each vitelline field and the pharynx. These gland cells stain more deeply than those of the anterior glands. The intestine is bifid, each crus being wide and largely overlain by the vitellaria. The presence of caeca could not be determined. The two crura appear to be joined just behind the testis. Whether there are other intercrural connections cannot be determined.

There are many rather large caudal glands in the stalk-like part of the body between the posterior limit of the vitellaria and the disc, the arrangement resembling that occurring in *Tetrancistrum sigani* and *Lintonia papillosa*. There are two pairs of eyes, the anterior being smaller and further apart than the posterior. The latter lie just above the front border of the pharynx. From the band-like brain a large nerve passes backwardly on either side of the pharynx, but its further course was not followed.

There is a single large testis, almost circular, and apparently perforated by other tissue, such as occurs in some Capsalid trematodes. It lies at the mid-length, and measures 0·4 by 0·5 mm., these dimensions being probably much greater than the normal size. The vas deferens arises from its anterior border, and passes forwards as a narrow duct beside, and partly above, the ovary and shell glands; it crosses the uterus, and appears to enter a rounded organ, which is probably the penis sac. There are two spicules of about the same breadth, but one is long (0·68 mm.) and strongly curved, and becomes much narrowed towards the free extremity, while the other is almost straight, and measures 0·2 mm. in length. One end of the smaller is bifid, each part being expanded, and one of them is somewhat boot-like, but the sac is so distorted that the figures may not represent the true relationship of the sac, spicules, and prostate ducts to one another. In the specimen, the narrow pointed tip of the larger spicule is sharply curved around the extremity of one of the outer ends of the smaller spicule. The genital aperture is median, just behind the bifurcation of the intestine. Prostate glands are abundant, the anterior and posterior groups being arranged apparently as in *Tetrancistrum*. The duct or reservoir of the posterior group contains granular material somewhat like that in the yolk ducts.

The ovary is rounded, measures about 0·22 by 0·18 mm., and lies immediately in front of the testis, a little to the right of the mid-line. The oviduct arises ventrally, is joined by the vitelline duct almost at once, and then passes forwards in a curved course to receive the ducts of the shell gland in front of the ovary. It then travels anteriorly as a thin-walled tube, terminating beside the male pore. The shell glands are situated in a narrow region in front of the ovary and between it and the vitelline fields.

The vagina opens on the ventral distance some distance postero-laterally from the genital pore, not far from the right margin of the parasite. The aperture is an oblique slit leading into a rather large cavity, or bursa vaginalis (Goto, 1), which is elongated in the direction of the slit. The bursa is surrounded by circular and radiating muscles and by gland cells. A very narrow vaginal canal passes from it inwardly and slightly backwards below the vitelline follicles, but when near the ovary it bends to enter the ootyp just before the latter receives the shell glands. A small sac-like structure with chitinized walls seems to be present at the point of junction, and may perhaps represent a seminal receptacle.

The vitellaria occupy the lateral fields in the broadened portion of the parasite, and extend almost from the level of the sex ducts to the anterior limit of

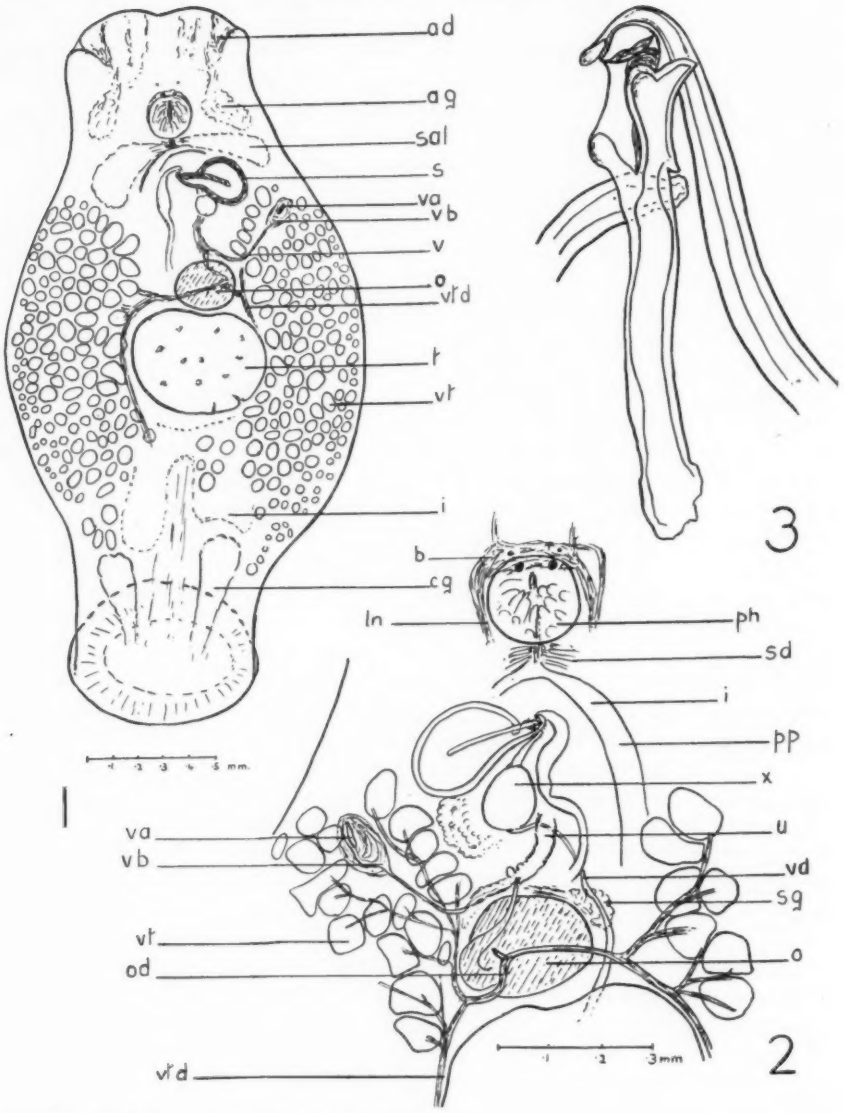

Fig. 1. Entire compressed specimen, dorsal view.

Fig. 2. Genital system, brain, etc., ventral view.

Fig. 3. Smaller spicule and the extremities of the longer spicule, ventral view.

Reference to lettering:—ad, anterior depression; ag, anterior glands; b, brain; cg, caudal glands; i, intestine; ln, longitudinal nerve; o, ovary; od, oviduct; ph, pharynx; pp, posterior prostate duct; sal, salivary glands; sd, salivary ducts; sg, shell glands; sp, spicules; t, testis; u, uterus; v, vagina; va, vaginal aperture; vb, vaginal bursa; vd, vas deferens; vt, vitelline follicles; vtd, vitelline duct; x, sac belonging to ejaculatory apparatus or perhaps metraterm.

the caudal glands, leaving the mid-region free. They consist of numerous rather large follicles, varying in size (under compression) from 0·05 by 0·05 mm. to 0·12 by 0·10 mm. They lie dorsally to the intestine. Each gives off a relatively wide, shallow duct inwardly, the various ducts occupying a ventral situation, and joining up to form eventually a duct which is connected with its fellow of the opposite side by a transverse duct lying ventrally to the ovary. From this canal a short common duct passes forwards, and soon joins the oviduct. An egg is not present in the specimen.

The only species of the genus, so far known, seems to be *A. richiardii* Sonsino (6), of which three specimens were collected in 1889 from the gills of a fish, *Pagrus orphus*, in the Mediterranean. The original account is very brief and unfigured. The genus was diagnosed as possessing anteriorly two bothria rather than suckers; subterminal mouth; four eyes; sucker-like, unarmed posterior disc; single testis; male aperture median, with spicule; vaginal opening on the left. The parasites, which measured 5 to 7 mm. by 1·3 mm., were regarded as lying between the Tristomids and the Gyrodactylids, and differing from the former in possessing a wide intestine. St. Remy and Perrier regarded the species as a member of the Tristomidae, Udonellinae, while Monticelli (1892) placed it under Calceostominae. The latter author (5) re-examined Sonsino's material, added some additional details regarding structure, and gave a few figures. He was unable to detect the aperture which Sonsino regarded as that of the vagina. He stated that the intestine appeared to be a very elongate single sac. The vitellaria were indicated as small and very numerous, distributed from the level of the genital apertures almost to the insertion of the posterior sucker. He placed the genus, along with *Merizocotyle*, *Dionchus*, *Lophocotyle*, and *Lintonia*, in the Monocotylidae, Anisocotylinae. Pratt listed it amongst the Gyrodactylidae.

Johnston and Tiegs, in their attempt to reclassify the Gyrodactyloidea (2), referred to the genus as possibly related to the Calceostomidae, Dionchinae, and removed it from the other genera mentioned by Monticelli. A provisional generic diagnosis was suggested by them, but this may now be emended as follows: Body fusiform, with truncate extremities; posterior disc sucker-like, round, unarmed; four eyes; glands opening into a depression on either side of the anterior extremity; male and uterine pores median and ventral, just behind the pharynx; two male spicules; single testis; ovary in front of testis and slightly to one side of the median line; vagina opening ventrally on the right; vitellarium consisting of numerous follicles lying laterally; characters of intestine not satisfactorily known—probably with wide crura and short caeca, and with intercrural communication behind the testis and perhaps elsewhere.

It is of interest to note that the host in each case is a member of the Sparidae (sea breams). *A. australis* differs from *A. richiardii* in its dimensions, the positions of the testes and ovary in relation to body length, and the size and distribution of the vitellaria.

A. australis exhibits a striking similarity to *Tetrancistrum sigani* Goto and

Kikuchi (1917), in form and in most of its anatomical features. The general topography of the male and female systems is almost the same in the two parasites. The main differences concern the male spicules and the vitellaria, which in the Japanese species are not divided up into separate follicles. The anterior region does not possess a sucker-like glandular depression, though the authors state that the lobes present are protrusible. It should be mentioned that in the specimen of *A. australis* the depression is inconspicuous. The posterior disc is similarly shaped, but is armed with a median series of hooks. The host is *Siganus fuscescens,* which belongs to a different suborder (Siganoidea) of the Percomorpha from that which includes the Sparidae. One species of this genus of fish, *S. nebulosus* Q. & G., occurs plentifully in Sydney Harbour.

Monticelli's genus *Lintonia,* as diagnosed by him (4), would fall under *Anoplodiscus,* though there is a possibility that the characters of the intestine may be quite different. Linton's figures (3) of the type species, *L. papillosa* (Linton) from *Gadus,* are merely rough sketches, but they indicate the presence of a very long eversible cirrus and, apparently, communication between the two crura. Monticelli states that the intestine is sac-like, and his figures show great similarity to those of *Anoplodiscus.*

Johnston and Tiegs were not able to consult Monticelli's account of the genus, but they noted that Linton's figures suggested that the species belonged to the Gyrodactylidae. A study of Monticelli's paper allows one to assign the parasite to the family definitely, as Poche (1928) has done recently. It appears justifiable to regard both *Lintonia* and *Anoplodiscus* as belonging to the subfamily Tetraonchinae, though they do not possess the hooks normally present on the disc of members of the Gyrodactylidae. The diagnosis of the family and subfamily given by those authors would then need slight emendation regarding the characters of the disc in order to receive such genera. *Tetrancistrum* had been assigned previously to the same subfamily by them.

The type slide of *A. australis* has been deposited in the South Australian Museum collection.

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THE COLORIMETRIC ESTIMATION OF NITROGEN BY "DIRECT NESSLERIZATION," WITH A NOTE ON A MODIFIED NESSLER-FOLIN REAGENT

by

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INTRODUCTION.

In view of the comparatively large number of papers dealing with the colorimetric estimation of nitrogen which have appeared in the literature during recent years, the author feels that some apology is necessary for presenting a fresh communication on the subject.

The modifications of the usual nesslerization procedure to be described have proved so satisfactory for the specific purpose for which they were designed, namely, the estimation of nitrogen in barley and malt and other material having a high carbohydrate-nitrogen ratio, that it seemed desirable to publish them.

When one attempts to apply the original direct nesslerization method of Folin (2), which succeeds admirably with physiological fluids comparatively rich in nitrogen, such as blood and urine, to substances containing but a small percentage of nitrogen and rich in carbohydrates, one immediately encounters difficulties. The amount of acid digestion mixture necessary to effect oxidation of the material, in a reasonable time and without excessive frothing in the initial stages of the digestion, has to be so increased that the concentration of salts finally appearing in the diluted and nesslerized digest is such as to cause inevitable clouding of the solution, and so render accurate colorimetric comparison impossible. If the digest is diluted before nesslerization to such an extent that clouding does not occur, the colour developed may be so weak that the inherent personal error in reading the colorimeter is such as to affect seriously the accuracy of the final results. It is thus seen that there is a certain limiting ratio of free acid to nitrogen in the diluted digest, which may not be exceeded if satisfactory results are to be obtained. By the employment of the special digestion mixtures of high oxidizing power which have been proposed from time to time (for instance, those involving either the use of hydrogen peroxide (3) or perchloric acid (5), or a combination of these reagents (4), it is possible to keep within the limits of this

ratio. It may be stated, however, without going into actual experimental details, that the author has proved to his satisfaction that when applying such methods to materials of the nature under consideration in this paper there is a danger of a serious loss of nitrogen, presumably caused by oxidation of some ammonia to free nitrogen. It was always found that higher results were obtained when the oxidation was carried out with a mixture of sulphuric acid and potassium sulphate according to the regular macro-Kjeldahl procedure. A definite improvement in direct nesslerization technique was made by Chiles (1), who made use of a protective colloid solution to inhibit the precipitation of the colloidal complex responsible for the colour of nesslerized solutions. By this means nesslerization can be satisfactorily effected in the presence of a fairly high concentration of alkali sulphates, but here again the method breaks down when the use of more than 0·5 cc. of concentrated sulphuric acid per 0·5 mgm. of nitrogen is necessary for the oxidation of the material. The author has found that by using an improved protective colloid solution and a modified Nessler-Folin reagent in which lithium hydroxide replaces the customary sodium hydroxide, it is possible to nesslerize in a total volume of 100 cc. solutions containing 2 cc. of concentrated sulphuric acid and up to 1 mgm. of nitrogen. The nesslerized solutions remain clear for weeks.

PREPARATION OF THE REAGENTS.

1. *Protective Colloid Solution.* 100 gm. of gum ghatti are suspended in a muslin bag in 300 cc. of water for a few days. A portion of the gum dissolves, giving a viscous solution; the remainder, together with any dirt, etc., remains in the bag as a thick gel. This latter is discarded, and the solution poured slowly with stirring into two volumes of alcohol. A sticky mass is precipitated, which may be easily collected on the end of the stirring-rod and placed into strong alcohol to harden. When sufficiently hard the precipitated gum is removed from the alcohol, coarsely crushed, and allowed to air dry. It is then finely powdered, and a 2% solution in ammonia-free thymol-saturated water prepared. This solution, which is perfectly stable, is used in the proportion of 3 cc. to every 100 cc. of final nesslerized solution. It possesses a "protective" action as measured against a gold sol of approximately 12 times that of Chile's solution, which is prepared from gum arabic.

2. *The Modified Nessler Reagent.* A quantity of saturated lithium hydroxide[1] solution is prepared by boiling an excess of the compound with water for some time, allowing to cool, and then filtering through a pad of asbestos in

[1]Supplies of lithium hydroxide were not available in Australia at the time of publication of this paper. The compound may be prepared, however, by treating a concentrated lithium sulphate solution with slightly less than the equivalent amount of barium hydroxide in the form of a hot saturated solution. The precipitated barium sulphate is filtered off, and the filtrate boiled down in a silver vessel to crystallizing point.

a Buchner funnel. The resultant solution contains about 12·3% of LiOH. The only commercial varieties of lithium hydroxide which the author has had an opportunity of examining (Hopkins and Williams' "pure" and Merck's "Lithium-hydroxid Kristallisiert" 53% LiOH) contain a trace of some substance, probably barium, which gives rise to a slight turbidity on neutralization with sulphuric acid. In order to avoid this turbidity appearing in the final nesslerized solutions the saturated LiOH solution is treated with a few drops of sulphuric acid, allowed to stand until the precipitate settles out, and then either decanted or filtered. To 1,000 cc. of this solution are added 200 cc. of the potassium mercuric iodide reagent, as prepared by Koch and McMeekin (3). The mixture is allowed to stand until the slight precipitate settles out, and the practically colourless supernatant liquid is then decanted off for use. The alkalinity of this reagent is such that about 21 cc. will neutralize 2 cc. of concentrated sulphuric acid; an extra 4 cc. will develop the full colour obtainable with amounts of nitrogen up to 1 mgm. It is therefore used in the proportion of 25 cc. per 100 cc. of final nesslerized volume.

On account of its cost it is not proposed that for ordinary work this reagent should take the place of the usual Nessler-Folin reagent. It should be noted also that its use is inapplicable in those cases where digestion mixtures containing phosphoric acid have been used, owing to the precipitation of lithium phosphate which would take place on nesslerization. On the other hand, the use of the protective colloid solution would appear to be generally advantageous, on account of the great stability it confers on nesslerized solutions.

The author's mode of procedure when using these reagents may be exemplified by citing the methods used in this laboratory for the determination of total nitrogen in barley (containing approximately 1·2–2·0% nitrogen) and in malt wort (carbohydrate-nitrogen ratio about 140 : 1).

(a) *Barley.* 1 gm. of the barley is weighed into a 100 cc. pyrex Kjeldahl flask, 20 cc. of concentrated sulphuric acid and a small crystal of copper sulphate added, and the mixture heated over a micro-burner until all particles of carbon have been washed down from the sides of the flask and the acid is refluxing gently (about 30 minutes). The contents of the flask are allowed to cool somewhat, 10 gm. of potassium sulphate are added, and the materials then gently boiled until pale green in colour. The total time of digestion is from 1 to 1½ hours. After cooling, the contents of the flask are made up to 100 cc. with ammonia-free water. Five cc. of this solution (equivalent to 0·05 gm. of barley, and containing from 0·6–1·0 mgm. of nitrogen) are delivered from a standardized pipette into a 100 cc. volumetric flask, and diluted to about 60 cc. with ammonia-free water. Three cc. of the protective colloid solution are then added, followed by 25 cc. of Koch and McMeekin's modified Nessler-Folin reagent, which is run in (conveniently from an automatic pipette) while gently rotating the flask.

The solution is then diluted to the 100 cc. mark. Simultaneously with the unknown a standard is prepared, containing 1 cc. of concentrated sulphuric acid, 3 cc. of the protective colloid, and a suitable amount of standard ammonium sulphate solution (10 cc. = 1 mgm. of nitrogen), together with 25 cc. of Nessler reagent. The volume of ammonium sulphate is so adjusted that when the standard is set at 20 mm. in the colorimeter the reading of the unknown will be within the limits of 17·5–22·5 mm.

(b) *Malt-Wort.* One cc. of the wort (containing approximately 0·5 mgm. nitrogen) is measured from an Otswald pipette into a pyrex test tube (8 in. x 1 in.), and 2 cc. of concentrated sulphuric acid added, together with a trace of copper sulphate. The mixture is cautiously heated over a micro-burner until all the moisture has been driven off and the acid is gently refluxing. About 1 gm. of potassium sulphate is added, and the boiling continued until the fluid is pale green. (Total digestion time about 30 minutes.) After cooling, the contents of the tube are washed into a 100 cc. flask and diluted to about 60 cc. The nesslerization is then effected as described under "barley," with the exceptions (owing to the relatively larger amount of sulphuric acid used in the digestion) that the author's modification of the Folin-Nessler reagent is employed, and 2 cc., instead of 1 cc., of concentrated sulphuric acid are added to the standard.

It is hoped that these examples will be sufficient to enable other workers to adapt the use of these reagents to suit their particular needs.

In conclusion, the author's thanks are due to Mr. T. A. Nation (Chief Brewer), and to the Directors of the Company, for permission to publish these results.

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THE ACTION OF DILUTE FORMALDEHYDE SOLUTION ON PROTEINS AND PROTEIN DERIVATIVES

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The use of dilute formaldehyde in the production of bacterial anatoxins for immunizing purposes attaches considerable importance to this reaction. Formolized bacterial toxins have been used for many years, but the mechanism of the change from toxin to anatoxin has not yet been elucidated. It is with this end in view that this work has been undertaken. Glenny and Hopkins (4) and Ramon (8) incubated diphtheria toxin with dilute formaldehyde. Its toxicity was thereby greatly diminished, but its power of inducing the formation of antibodies to the original toxin was unimpaired. According to Watson and Langstaffe (10) there is a decrease in the amino nitrogen content of the solution during this treatment. Martin (7) reduced the toxicity of the venoms of the Cobra and Daboia by similar treatment.

Most experimental work concerning the action of formaldehyde on proteins has involved the use of more concentrated solutions of formaldehyde than is characteristic for this reaction. A few investigators have worked with dilute solutions. Blum (3) found that diluted egg white will not coagulate on heating after the addition of a few drops of 40% formaldehyde solution. Schwarz (9) obtained similar results with various proteins, and showed that coagulation could be brought about by the addition of salts. Benedicenti (2) investigated the quantitative combination of formaldehyde in dilute solution with several proteins over a period of two to three weeks. Henley (5), working with blood serum, observed a slow progressive decrease in the solubilities of the proteins in ammonium sulphate solution after the action of formaldehyde in as low a concentration as 0·09%. The results of these workers indicate a definite alteration of the properties of the proteins by the action of dilute formaldehyde. The experiments described in this paper consist of some attempts to study the effects of a similar reaction on certain proteins and on their disintegration products by enzymes, by an investigation of the changes of amino nitrogen content and specific rotation after incubation at 37°C. in a solution containing 0·5% formaldehyde.

Experimental Procedure.

Neutralized commercial formalin was added to the solutions, its formaldehyde content being taken as 40%. Toluene was added in all cases as a preservative. The reaction of the proteose and peptone solutions was from pH 6·5 to pH 6·0. Amino nitrogen was estimated with a micro Van Slyke apparatus. Light of wave length 5461 A.U.[1] from a mercury arc lamp was used as the light source for polarimetric readings. The concentration of the experimental solutions was estimated in duplicate by evaporation of 2 cc. or 5 cc. of the solution in a porcelain crucible on a boiling water bath, with subsequent drying in an air oven at 100–105°C. The concentrations of the solutions were usually about 2%.

The proteins used, with the exception of gelatine, were prepared in this laboratory. As a convenient source for proteoses and peptone, "Berna" commercial peptone was first used. There was difficulty in achieving complete separation of the metaprotein from the rest of the solution. The pH was adjusted to pH 5·8 and after heating the metaprotein was filtered off. The primary and secondary proteoses were separated by precipitation with half and full saturation with ammonium sulphate. After washing, the proteoses were dissolved and reprecipitated. The pH of the solution of primary proteose was adjusted to 5·8, and a further amount of metaprotein which settled out was separated by filtration. Even after this treatment a small percentage of metaprotein is present in the primary proteose fraction. This is filtered off from the soluble primary proteose and ammonium sulphate, when the solid precipitated by alcohol is dried and then treated with distilled water. The primary proteose was further fractionated by precipitation with 50% and 80% alcohol. On dialysis of the primary proteoses a viscous precipitate settles which is soluble in dilute acid and alkali. This was at first thought to be metaprotein. However, it was found to be soluble in sodium chloride solution and not coagulable on heating, and is evidently a very similar body to the heteroproteose of the earlier literature. The peptone was obtained from the filtrate after full saturation with ammonium sulphate by first evaporating to dryness *in vacuo*, and then extracting the peptone from the solid ammonium sulphate with hot 95% alcohol. This solution was then concentrated to small volume *in vacuo*, and the peptone precipitated by the addition of absolute alcohol and ether and dehydrated by stirring with more alcohol. Peptone prepared in this manner contained a high percentage of chloride from the hydrochloric acid added before peptic digestion. The chloride was removed by precipitation with silver sulphate, the sulphate being precipitated by baryta. In later experiments sulphuric acid was used for adjusting the pH of the fibrin suspension for peptic digestion, and the peptone thus obtained was free from salts. All the fractions except peptone were dialysed to free them from ammonium sulphate. Some loss of proteoses during dialysis could not be pre-

[1] A.U.=Ångström Unit.

vented. Pyroxilin membranes prepared by Adair's (1) method were used. Even after drying for five minutes between each of six coats, and then finally for twenty minutes, the membranes were somewhat permeable to them.

As it was desired to work with proteoses and peptones derived from individual proteins, some were prepared from crystalline hen egg albumin and from ox fibrin by peptic digestion, and separated as were the "Berna" proteoses, except that the primary proteoses were not further fractionated by alcohol. A small amount of heteroproteose was obtained from egg albumin and a larger quantity from fibrin.

The tryptic digests were prepared by incubation for ten days with trypsin. They were directly treated with formaldehyde after neutralization and heating to inactivate the enzyme.

Results for Native and Denatured Proteins.

The effect of 0·5% formaldehyde was first tried on solutions of native and denatured proteins. Optical rotations were observed using light of wave length 5461 A.U. except in the case of globin when wave length 5780 A.U. was employed. The solutions were incubated and readings taken during five to seven days. There was no definite change in optical activity and no precipitate. The native euglobulin solutions were too opalescent for optical readings. Opalescence increased in them as the experiment proceeded. The other solutions remained clear. The optical rotation of the native proteins did not increase, showing that no denaturation had occurred (6). The solutions treated with formalin gave similar reactions to Millon's tryptophane biuret and Molische's tests as the control protein solutions.

TABLE 1.

Optical Rotations Observed for Protein Controls and for Proteins Treated with Formaldehyde.

Protein.	Optical reading Control.	Optical reading after formaldehyde treatment.
A. Dissolved in water.		
(i) Native ox serum pseudoglobulin	—1·90°	1·92°
(ii) Crystalline egg albumin	—0·60°	—0·60°
(iii) Gelatine	—0·73°	—0·73°
B. Dissolved in sodium chloride solution.		
(i) Native ox serum euglobulin	Opalescent	Opalescence increased
(ii) Native horse serum euglobulin	"	" "
C. Dissolved in dilute alkali.		
(i) Casein	—1·20°	—1·20°
(ii) Denatured serum euglobulin	—0·61°	—0·63°
(iii) Denatured serum pseudoglobulin	—0·65°	—0·65°
(iv) Denatured serum albumin	—0·42°	—0·42°
(v) Denatured ox globin	—0·56°	—0·56°
D. Dissolved in dilute acid.		
(i) Denatured ox globin	—0·61°	—0·62°

The change in amino nitrogen concentration after incubation with formaldehyde for ten days was later determined for casein, gelatine, and some denatured proteins. Van Slyke determinations were not attempted in the case of native proteins as it was considered that the results so obtained would not be conclusive evidence of any specific reaction. The denaturation of the protein during the estimation might be responsible in part for any changes observed. In dilute acid the denatured proteins showed a tendency to gel formation, especially in the case of egg albumin. There was also an increased opalescence, and to make polarimetric readings possible the solutions of casein were heated with a few drops of 10% acetic acid on a boiling water bath. This may account for the observed changes in optical activity. In alkaline solution the proteins did not form gels nor become opalescent. In all the proteins on which Van Slyke determinations could be made there was a decrease in amino nitrogen content after incubation with 0·5% formaldehyde. During the period of incubation there also seems to be some slow hydrolysis of the proteins especially in acid solution, as the amino nitrogen content of the controls increases during the experiment. The last two proteins recorded had a shorter period of incubation than the rest, and the amino nitrogen concentrations of the control solutions are lower than those of the other control solutions. The results are shown in Table 2.

TABLE 2.

Results for Proteins—Observations of Specific Rotations and Amino Nitrogen Contents.

Protein.	Control.			After treatment with 0·5% formaldehyde for 10 days.		
	Concentration.	Specific Rotation.	% amino nitrogen.	Concentration.	Specific Rotation.	% amino nitrogen.
A. Dissolved in water.						
Gelatine	2·25%	−156·8°	0·52 0·52	2·26%	−155·3°	0·35 0·36
B. Dissolved in acid.						
Denatured horse globin	0·935%	−55·6°	0·92 0·85	1·02%	−50·9°	0·40 0·45
Casein	1·82%	−98·9°	0·76 0·77	1·75%	−82·3°	0·44 0·45
C. Dissolved in alkali.						
Denatured egg albumin	1·21%	−55·3°	0·35 0·36	1·23%	−55·3°	0·13 0·11
"Berna" metoprotein	1·62%	Too opalescent to read	0·83 0·81	1·58%	Too opalescent to read	0·19 0·24
Casein	2·0%	—	0·34 0·31	2·0%	—	0·18
Denatured horse globin	2·0%	—	0·23 0·26	2·0%	—	0·16

Results for Proteoses, Peptones and Tryptic Digestion Products.

The proteose, peptone and tryptic digestion products were incubated for ten days with formaldehyde. One lot of proteose was left for three weeks, but the loss of amino nitrogen was no greater than that occurring in ten days. In the case of the primary proteoses investigated on the addition of formaldehyde a precipitate appears in a few hours, and slowly increases in amount over a period of several days. This precipitate is insoluble in water and cold dilute acid or alkali, and is digestible by pepsin and trypsin. It dissolves when heated for a short time on a boiling water bath with N/10 sodium hydroxide or N/2 acetic acid. The time required for solution varies from twenty to fifty minutes, according to the amount of precipitate. This insolubility of the precipitate in cold acid or alkali appears to be merely due to a secondary effect of the formaldehyde. A suspension of metaprotein is similarly rendered insoluble if left standing in 0·5% formaldehyde, and heating is required to obtain its solution. Also if a denatured protein is incubated with formaldehyde in acid solution at a pH near that of its isoelectric point an insoluble precipitate appears. This does not occur if the solutions are sufficiently acid or alkaline. If the precipitate obtained from a primary proteose is filtered off as soon as it precipitates it is soluble in both dilute acid or alkali in the cold. It possesses the properties of a metaprotein with an isoelectric point at pH 5·2 to 5·4 for "Berna" metaprotein. The isoelectric point is more acid in the case of egg albumin. On incubation of the proteose solution with formaldehyde at a more acid or alkaline pH the precipitate does not appear until the isoelectric point is approached. After solution of this precipitate by heat, its metaprotein character is partly or completely destroyed. On neutralization only 15%, or even less, of the "Berna" metaprotein precipitates.

The whole of the primary proteose is not precipitated by formaldehyde. Approximately 50% of the first primary proteose fraction, 70% of the second primary proteose fraction of "Berna" proteoses, 60% of the fibrin primary proteose, 40% of the egg albumin primary proteose, and 25% of the secondary proteose of egg albumin appear as precipitates. After incubation for ten days this precipitate was filtered off and washed well in water before dissolving. The filtrate was kept for separate investigation. In a few cases it needed dilution for optical readings, owing to opalescence. A decrease in amino nitrogen content was observed in both precipitate and filtrate. The peptones and tryptic digestion products gave no precipitate on similar treatment. The peptones and tryptic digests are the only solutions showing a marked change in specific rotation after treatment with formaldehyde.

The results are given in Table 3. Substances numbered 1, 2, 4, 5, and 8 were separated from one sample of "Berna" commercial peptone, while those numbered 3, 6, 7, 9, and 10 were from a second sample. The egg albumin digestion products were all obtained from one digest. The experiment was carried out with incubation for three weeks in the case of numbers 11 and 13, and for the usual

time of ten days for numbers 12 and 14. Two portions of the same preparation of ox fibrin heteroproteose were used in experiments 16 and 17. Two separate preparations of fibrin products were made, numbers 18 and 20 belonging to one batch, and 19 and 21 to the other. The peptone, 22, was prepared separately from a digest acidified with sulphuric acid instead of the usual hydrochloric acid.

The presence of formaldehyde at the end of the reaction was ascertained by the resorcinol test. In all cases there was some present after the ten days duration of the experiment.

DISCUSSION.

Although there is not yet sufficient evidence to indicate the ultimate nature of the reaction of formaldehyde on proteins and on the products of their hydrolysis by enzymes, there are one or two points among the data presented here which are worthy of consideration. It seems quite certain that some special grouping of the protein molecule is involved. The whole of the amino nitrogen does not react, since the protein still contains amino nitrogen after prolonged formaldehyde treatment. If the reaction concerned only the free amino groups of the molecule, one would expect the loss of amino nitrogen to be greater in the case of the tryptic digestion products than in that of the proteoses since more free amino groups are freed as digestion proceeds. However the proportional decrease of amino nitrogen is greater in the case of the proteins and proteoses.

It might be thought that the metaprotein-like precipitate which appears in the proteose solutions when these are treated with formaldehyde is not a product of the reaction, but was already present in the proteose solution, and was prevented from separating out because of its association with the proteose. Since no precipitate separates out either through dilution of the proteose solution or variation in its pH over a range from 2 to 10, and since no coagulum is formed on boiling, this view is unlikely to be correct.

An increase of amino nitrogen content of the control solutions was observed during incubation. This would appear to be caused by a slow hydrolysis. During a period of seven days the amino nitrogen content of "Berna" secondary proteose had increased from 0·7% to 0·9%, of egg albumin secondary proteose from 1·0% to 1·2%, and of fibrin secondary proteose from 0·7% to as much as 1·3%. This fact accounts for the irregularity of the amino nitrogen content of the control solutions.

It is remarkable that before the chloride was removed from the peptone solutions there was no reaction between the peptone and formaldehyde, there being no change of amino nitrogen content or of specific rotation. When the heteroproteose is incubated in sodium chloride solution the decrease of amino nitrogen is as great as after incubation in very dilute acid. In the latter case all the proteose is precipitated as are denatured proteins if treated with formaldehyde near their iso-electric points.

TABLE 3.

Results for Proteoses, Peptones and Tryptic Digests—Observations of Specific Rotations and Amino Nitrogen Contents.

		Control.			Filtrate from precipitate after treatment with 0·5% formaldehyde.			Precipitate obtained by treatment with 0·5% formaldehyde.			
		Concentration.	% amino nitrogen.	Specific rotation. λ=5461 A.U.	Concentration.	% amino nitrogen.	Specific rotation. λ=5461 A.U.	Concentration.	% amino nitrogen.	Specific rotation. λ=5461 A.U.	
"Berna" primary proteose, precipitated by 50% alcohol	1	1·50%	0·89	—76·8°	1·33%	0·27	—67·4°	0·53%	0·33	—77·3°	
						0·28					
	2	3·38%	0·86	—79·3°	1·29%	0·33	—88·8°	0·74%	0·43	—74·4°	
			0·84			0·36			0·48		
	3	1·94%	0·80	—76·8°	0·84%	0·20	—76·2°	1·46%	0·28	—79·5°	
			0·81			0·19			0·34		
"Berna" primary proteose, precipitated by 80% alcohol	4	2·64%	0·72	—70·3°	0·43%	0·73	—72·4°	3·32%	0·47	—78·3°	Incubated only until precipitate settled, 4 days
			0·71			0·72			0·49		
	5	1·67%	0·75	—89·9°	0·61%	0·68	—87·2°	1·37%	0·41	—84·6°	Incubated only until precipitate settled
			0·65			0·80			0·48		
	6	2·51%	0·76	—73·1°	0·62%	0·72	—88·7°	3·72%	0·43	—84·5°	
			0·78			0·73			0·42		
					Left for one week longer with formaldehyde.						
						0·35	—81·5°				
						0·27					
	7	5·45%	0·79	—79·8°	1·70%	0·35	—76·6°	2·71%	0·53	—77·1°	
			0·75			0·36			0·50		
"Berna" secondary proteose	8	1·16%	0·87	—75·4°	1·14%	0·24	—76·2°				Trace of precipitate appears
			0·91			0·28					
	9	2·09%	1·02	—80·9°	2·05%	0·44	—76·1°				
			0·99			0·48					
'Berna" peptone	10	2·27%	2·08	—57·7°	2·30%	1·05	—64·4°				No precipitate
			2·11			1·08					
Egg albumin primary proteose	11	1·43%	1·24	—87·4°	0·87%	0·47	—85·1°	1·17%	0·54	—79·7°	
			1·22			0·42			0·67		
	12	2·13%	0·84	—86·8°	1·02%	0·39	—74·5°	0·73%	0·37	—84·4°	
			0·88			0·37					
Egg albumin secondary proteose	13	2·16%	1·30	—83·3°	1·62%	0·48	—	0·80%	0·22	—	Filtrate too opalescent for optical reading
			1·27			0·40			0·18		
	14	2·28%	1·04	—78·9°	0·76%	0·49	—96·5°	0·58%	0·21	—91·4°	
			1·03			0·52			0·21		
Egg albumin peptone	15	2·02%	1·91	—47·2°	1·94%	0·98	—51·5°				No precipitate
			1·93			0·98					
Ox fibrin heteroproteose (in acid)	16	2·79%	0·73	—95·4°				1·15%	0·22	—	Too opalescent for optical reading
			0·74						0·24		
Ox fibrin heteroproteose (in NaCl solution)	17	0·95%	0·73	—	0·95%	0·16	—	—	—	—	No precipitate
Ox fibrin primary proteose	18	3·58%	0·61	—99·7°	1·28%	0·12	—96·9°	2·34%	0·39	—94·0°	
			0·64			0·16			0·40		
	19	2·42%	1·08	—97·2°	0·80%	0·53	—95·0°	0·64%	0·45	—88·2°	
			1·03			0·55			0·42		
Ox fibrin secondary proteose	20	2·93%	1·21	—90·4°	2·27%	0·65	—	0·78%	0·40	—	Filtrate too opalescent for optical reading.
			1·22			0·67			0·36		Not sufficient precipitate for optical reading.
	21	2·70%	1·27	—92·3°	2·15%	0·59	—97·7°	0·72%	0·40	—81·9°	
			1·32			0·59					
Ox fibrin peptone	22	2·26%	2·73	—45·6°	2·40%	1·20	—54·6°				No precipitate
			2·77			1·23					
Egg albumin metaprotein tryptic digest	23	3·24%	7·15	—14·8°	3·28%	5·10	—21·3°				No precipitate
			7·25			5·05					
"Berna" primary proteose tryptic digest	24	2·91%	6·97	—31·3°	3·00%	4·60	—38·6°				No precipitate
			7·05			4·54					
Fibrin peptone tryptic digest	25	2·34%	6·75	—	2·40%	5·54	—				No precipitate
			6·80			5·35					

SUMMARY.

The action of 0·5% formaldehyde solution at 37°C. on certain proteins, proteoses, peptones, and tryptic digestion products has been investigated.

Forty per cent. to 70 % of the primary proteose solutions is precipitated on treatment with 0·5% formaldehyde. A smaller percentage of the secondary proteose solutions, 25% in the case of egg albumin secondary proteose, is also precipitated. This precipitate possesses the properties of a metaprotein.

A decrease in amino nitrogen content is observed after formaldehyde treatment in all the solutions investigated. The proportional decrease is greatest in the case of proteins and proteoses, and least in the case of tryptic digests.

The specific rotation of the peptone and tryptic digest solutions increases after formaldehyde treatment.

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OBSERVATIONS ON THE VENOM OF A LARGE AUSTRALIAN SNAKE, *PSEUDECHIS AUSTRALIS* (GRAY)

1. SYNONYMY

by

DONALD F. THOMSON.

(From the Walter and Eliza Hall Institute, Melbourne.)

(Submitted for publication 2nd August, 1930.)

Naja australis Gray, Zool. Misc. (1842), p. 55.
Pseudechis porphyriacus (part) Gunther, Cat. Colub. Snakes Brit. Mus. (1858), p. 218.
Pseudechis australis Gunther, Ann. Mag. Nat. Hist. (3), xii, p. 362 (1863); Krefft, Verteb. Lower Murray (1865), p. 32; P.Z.S., Lond. (1868), p. 3; Snakes of Aust. (1869), p. 47.
Pseudechis darwiniensis Macleay, P.L.S., N.S.W., ii (1878), p. 220 and iii (1888), p. 416.
Pseudechis cupreus (part) Boulenger, Cat. Snakes Brit. Mus. Nat. Hist. (1896), p. 329.
Pseudechis australis Boulenger, ibid., p. 330.
Pseudechis darwiniensis, ibid., p. 330.
Pseudechis cupreus Waite and Longman, Rec. South Aust. Mus. (1920), Vol. 1, No. 3, p. 175.
Pseudechis australis, ibid., p. 175.
Pseudechis darwiniensis, ibid., p. 175.
Pseudechis australis Kinghorn, J. R., Rec. Aust. Mus. (1924), xiv (3), p. 164; Waite, Rept. Amphib. S.A. (1929), p. 216; Kinghorn, J. R., Snakes of Aust. (1929), p. 99.
Pseudechis cupreus, ibid., p. 100.
Pseudechis darwiniensis, ibid., p. 104.

Much confusion exists in regard to the identity of several species of the genus *Pseudechis*. It originated in 1858 when Gunther failed to distinguish *P. australis* (a species established by Gray in 1842 as *Naja australis*) from the common black snake *P. porphyriacus*. Scarcely had this mistake been rectified when two further species were described, *P. darwiniensis* Macleay (1878) and *P.*

cupreus Boulenger (1896). The extreme confusion which exists in the literature dealing with these species is largely due to the comparatively small number of specimens available for examination and to non-recognition of the extreme variability of certain of the characters relied upon in distinguishing them. An examination of the situation suggests that *P. cupreus* rests in part upon an error in description of a specimen, and that *P. darwiniensis* is synonymous with *P. australis.*

The Genus Pseudechis.

Maxillary bone extending forwards as far as the palatine; two to five small solid teeth on the maxilla behind the poison fang; anterior mandibular teeth longer than the posterior.

All members of the genus have the body scales in 17 to 23 rows, and the number of transverse body scales has been used by Boulenger to separate the genus into two series. Later workers have also followed this classification. Records of specimens actually examined indicate that this character is constant and valid. Boulenger recognized four species in this series with 17 rows, i.e., *P. porphyriacus, P. cupreus, P. australis,* and *P. darwiniensis.* A fifth species, *P. denisonioides* Werner, has since been described and Waite and Longman (10), in revising Boulenger's key, include a sixth under the name of *P. porphyriacus,* var. *mortonensis,* the *P. mortonensis* of de Vis.

I am indebted to Mr. H. W. Parker of the Natural History Museum, South Kensington, for Gray's original description of *Naja australis* which was unobtainable in Australia.

> "Uniform pale brown, in spirits, rather paler below; anterior ocular short, broad, triangular, two posterior oculars square.
>
> "Inhabits North Coast, New Holland; Port Essington.
>
> "This scarcely differs from *N. haje* of Africa, except in having only two posterior ocular plates, and this may be only accidental in the individual."

Boulenger (1896) gives the following description:

> "Eye rather small, its diameter nearly equalling its distance from the mouth. Rostral broader than deep, visible from above; internasals about half as long as the prefrontals; frontal small, not or but slightly broader than the supra-ocular, once and two-thirds to twice as long as broad, as long as its distance from the rostral, two-thirds the length of the parietals; posterior nasal in contact with the single preocular; two postoculars; temporals 2–2, lower anterior large and wedged between the fifth and sixth supralabials; six upper labials, third and fourth entering the eye, three lower labials in contact with anterior chin shields, which are shorter than the posterior. Scales in 17 rows (19 or 20 on the neck). Ventrals 199 to 220; anal divided; subcaudals 57 to 70, 26 to 41 anterior single, rest

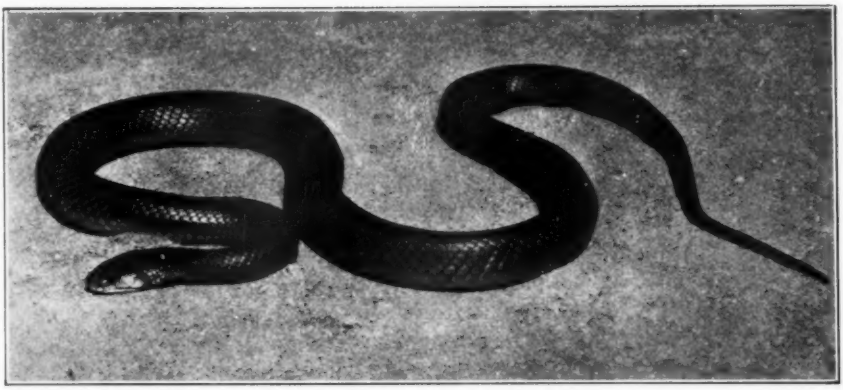

Figure 1. *Pseudechis australis* (Specimen No. 6, Table 1) from life.

in pairs. Uniform pale brown above, yellowish beneath. Total length 1080 mm.; tail 170 mm.; Northern Australia."

The material available for the examination of the species consists of five complete specimens and the head and neck of a sixth. Three of these specimens, together with the separate samples of venom obtained from two of them, were given me by Mr. W. McLennan, whom I had the good fortune to meet while carrying out field work on Cape York, in 1928. Mr. McLennan thought that these specimens were identical with two which he had obtained at Coen in 1922, which had been described by Kinghorn (1923), who thereby established a new genus and species, *Oxyuranus maclennani*. That one, at least, of McLennan's later specimens was not identical with *Oxyuranus maclennani* had been shown by Kellaway and Williams (4), for the venom of Kinghorn's snakes was wholly different in toxic constitution from that of one of the later specimens. The remaining specimens I collected on Cape York, and one of these I was able to keep alive for some months, collecting venom from it at frequent intervals.

Examination of these specimens convinced me that, though they exhibited some variation in external characters, they were all *P. australis*, an opinion with which Mr. Kinghorn of the Australian Museum, Sydney, who kindly examined them with me, concurred.

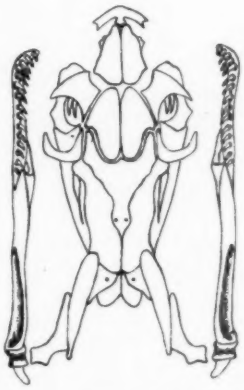

Figure 2. Dorsal view of skull of *Pseudechis australis* (Specimen No. 6, Table 1), natural size.

Figure 3. Lateral view of skull of *P. australis* (Specimen No. 6, Table 1).

Description of Pseudechis australis from Cape York Peninsula.

Owing to their close similarity a complete description is given of only one of the specimens collected in Cape York (Fig. 1). The localities and the characters of all the specimens are set out in Table 1.

Skull (two specimens) (Figs. 2 and 3).

Maxillary bone extending forward as far as the palatine. Poison fangs grooved, only slightly curved and 7·1 mm. in length, each with several well-developed reserve fangs, followed by three or four very small, solid teeth on the maxilla. Mandibles very strongly developed, the anterior teeth being slightly longer than the posterior, but less markedly so than in most *Demansia*.

TABLE 1.

Pseudechis australis Gray.

Number.	Locality.	Length, mm.	Tail, mm.	Width of head, mm.	Gape, mm.	Abdominal shields.	Subcaudal Shields.	Rostral. †B./L. = index.	Frontal. †B./L. = index.	Supraocular breadth.	Frontal / Supraocular = Index.	Distance from anterior tip of frontal to posterior border of rostral.
Cape York.												
1	Coen	1642	239	34	34·5	203	59 44 single 15 paired	9·5/5·5=1·73	10·5/7·0=1·5	5·5	7·0/5·5=1·27	10·5
2	Coen	1765	232	38·5	40	204	60 39 single 21 paired	11·5/6·5=1·77	9·0/7·5=1·2	6·5	7·5/6·5=1·15	11·5
3	Coen	1864	264	41·5	39·5	205	63 43 single 20 paired	10·5/6·5=1·62	11·0/7·5=1·47	7·0	7·5/7·0=1·07	11·0
4	Lower Coleman River	1700*	—	35·5	40	—	—	11·5/7·0=1·64	10·0/8·5=1·18	6·5	8·5/6·5=1·31	10·0
5	Lockhart River	1780	230	39	38	206	62 43 single 19 paired	11·0/6·0=1·83	9·5/6·0=1·58	5·5	6·0/5·5=1·09	9·5
6	Stewart River	2039	258	39	39	207	59 38 single 21 paired	9·5/7·5=1·27	11·5/7·0=1·64	6·5	7·0/6·5=1·08	—
Macleay Museum.												
1	Port Darwin	883	132	—	—	209	65 49 single 16 paired	5·5/3·5=1·57	6·5/4·5=1·44	3·0	4·5/3·0=1·5	—
2	Port Darwin	1384	205	—	—	208	62 37 single 25 paired	8·0/5·5=1·45	8·0/5·0=1·6	5·0	5·0/5·0=1·0	5·0
3	Port Darwin	1701	166	—	—	217	34 31 single 3 paired tail truncated	9·5/6·0=1·58	10·5/6·5=1·62	6·0	6·5/6·0=1·08	10·5

*Head only; length approximate. †B=breadth. L=length.
Body scales 17 and anal plate divided in all.

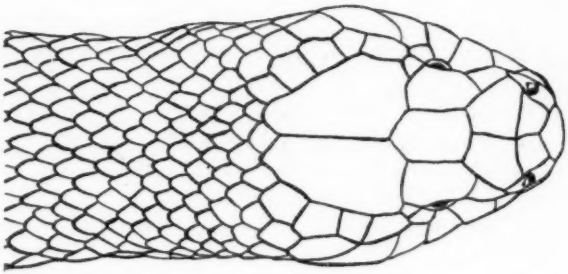

Figure 4. Dorsal surface of head of *P. australis* (Specimen No. 6, Table 1).

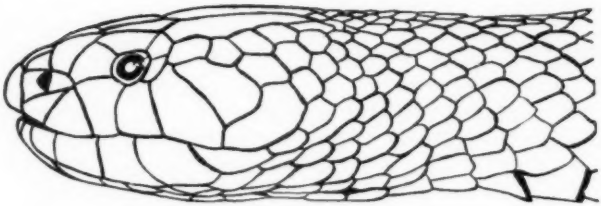

Figure 5. Lateral surface of head (Specimen No. 6, Table 1).

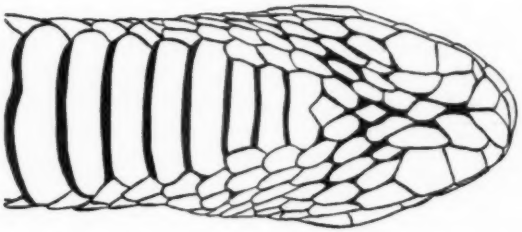

Figure 6. Ventral surface of head (Specimen No. 6, Table 1).

Figure 7. Subcaudal region (Specimen No. 6, Table 1).

General description (Figs. 4, 5, 6 and 7).

Locality, Stewart River, Princess Charlotte Bay; length, 2039·0 mm.; tail, 258·0 mm.; width of head, 39·0 mm.; gape, 39·0 mm.; body scales in 17 rows; abdominal plates, 207; anal plate divided; subcaudal scales, 59—38 single, 21 paired.

Head of great width, moderately distinct from body, wedge-shaped, and tapering at the snout. Eye moderately large, pupil circular, iris orange red in life. The width of head is due to the extremely large venom glands (21 by 11 mm.).

Rostral shield broader than long (9·5 by 7·5 mm.) and visible from above. Internasals (5·0 mm.), prefrontals (8·0 mm.), frontal (11·5 by 7·0 mm.) large, 1·6 times as long as broad, a little broader than the supraocular (6·5 mm.) and less than its distance from the tip of the snout (14·0 mm.), more than half the length of the parietal (20·0 mm.). Nasal in contact with single preocular, two postoculars. Temporals two to two, lower anterior deeply wedged between fifth and sixth supralabials. Supralabials, 6; third and fourth entering eye. Three anterior infralabials in contact with anterior chin shields, of which the anterior is shorter than the posterior.

Colour uniform olive brown after sloughing skin, becoming coppery brown later. Ventral surface yellowish white.

The description of this snake, No. 6 in table, as well as of those of the remaining five specimens tabulated, agrees closely with *P. australis*, except in regard to variations in the frontal shield measurements. Boulenger states that in *P. australis* the frontal shield is one and two-thirds to twice as long as broad, and not, or but slightly, broader than the supraocular. In only one specimen (No. 6) is the frontal fully once and two-thirds as long as broad, but even on this character the specimen cannot be separated from the remainder, which form a graded series in which the frontal is from 1·18 to 1·64 times as long as broad. In all the frontal is broader than the supraocular.

These variations place some of the specimens within the range of *P. darwiniensis*, which is differentiated by Boulenger, solely upon the fact that the frontal is approximately as broad as long, and broader than the supraocular.

An examination was therefore made of specimens in the Macleay Museum. Three specimens were described by Macleay (9), the type from Port Darwin and two further specimens from King's Sound, W.A. There are three specimens in the Macleay Museum for all of which the location given is Port Darwin. None agrees fully with the type description, and I was unable to ascertain the fate of the type. It may be assumed, however, that all three specimens were determined by Macleay. A summary of some measurements of these snakes is shown in Table 1. The six numbered specimens, together with the three from the Macleay Museum, form a graduated series between *australis* and *darwiniensis*, in which numbers 2 and 4 in the Cape York specimens more closely resemble Macleay's

TABLE 2.

Pseudechis porphyriacus Shaw.

Number.	Locality.	Number of abdominal shields.	Number of subcaudal shields.	Rostral shield. *B./L.=index.	Frontal shield. *B./L.=index.	Supra-ocular breadth.	Frontal / Supra-ocular =index.
1	♀ (Nat. Mus., Melb.)	182	51 10 single 41 paired	9·0/5·5=1·64	7·5/6·0=1·25	5·50	6·0/5·5=1·09
2	Victoria	182	53 11 single 42 paired	5·5/4·0=1·38	6·0/4·5=1·33	4·50	4·5/4·5=1·00
3	Victoria	175	53 7 single 46 paired	6·5/4·0=1·63	6·0/4·5=1·33	5·00	4·5/5·0=0·90
4	Herbert River, Queensland (Macleay Mus.)	—	53 12 single 41 paired	—	10·0/7·5=1·33	7·00	7·5/7·0=1·07
5	Purnong, Sth. Aust. (Nat. Mus., Melb.)	178	50 9 single 41 paired	3·5/2·5=1·40	4·0/3·0=1·33	2·50	3·0/2·5=1·20
6	Victoria	182	55 9 single 46 paired	6·0/4·5=1·33	7·0/5·0=1·40	5·00	5·0/5·0=1·00
7	Brigalow, Sth.-West Queensland	184	56 11 single 45 paired	7·5/4·5=1·67	7·0/5·0=1·40	5·00	5·0/5·0=1·00
8	Victoria	179	49 16 single 33 paired	7·5/4·5=1·67	7·0/5·0=1·40	5·50	5·0/5·5=0·91
9	Victoria	180	54 9 single 45 paired	7·5/5·0=1·50	7·0/5·0=1·40	5·50	5·0/5·5=0·91
10	Victoria	185	52 4 single 48 paired	6·0/5·0=1·20	7·0/5·0=1·40	4·50	5·0/4·5=1·11
11	Victoria	180	portion of tail lost	8·0/4·5=1·78	7·0/5·0=1·40	5·50	5·0/5·5=0·91
12	Herbert River, Queensland (Macleay Mus.)	—	—	—	8·5/6·0=1·42	5·50	6·0/5·5=1·09
13	♀ (Nat. Mus., Melb.)	188	56 8 single 48 paired	8·5/6·0=1·42	8·5/6·0=1·42	6·00	6·0/6·0=1·00
14	Rutherglen, Victoria (Nat. Mus., Melb.)	178	53 6 single 47 paired	6·5/4·0=1·63	5·0/3·5=1·43	4·50	3·5/4·5=0·78
15	Grafton, New South Wales	193	54 7 single 47 paired	7·5/5·5=1·36	8·0/5·5=1·45	5·50	5·5/5·5=1·00
16	Victoria	176	57 15 single 42 paired	7·5/5·0=1·50	7·5/5·0=1·50	5·00	5·0/5·0=1·00
17	Brigalow, Sth.-West Queensland	177	49 3 single 46 paired	6·0/4·0=1·50	6·0/4·0=1·50	4·00	4·0/4·0=1·00
18	♀ (Zool. Mus., Univ. of Melbourne)	184	54 10 single 44 paired	5·5/5·0=1·10	6·0/4·0=1·50	5·00	4·0/5·0=0·80
19	Victoria	180	51 11 single 40 paired	8·0/5·5=1·45	7·5/5·0=1·50	5·00	5·0/5·0=1·00
20	Herbert River, Nth. Queensland (Macleay Mus.)	—	—	—	7·5/4·5=1·67	4·00	4·5/4·0=1·13
21	Victoria	180	49 11 single 38 paired	7·5/4·5=1·67	7·5/4·5=1·67	5·00	4·5/5·0=0·90

*B=breadth. L=length.

Body scales 17 and anal plate paired in all.

type than the Port Darwin specimens. The absence of any striking distinction between the specimens in the Macleay Museum and those obtained on Cape York suggests that the character on which the two species were distinguished is not a valid one.

With the object of determining the value of the proportion between the length and breadth of the frontal shield, a series of 21 specimens of *P. porphyriacus,* the only member of the genus of which any considerable number of specimens could readily be obtained, was examined. These came from widely separated localities. The results of their examination, set out in Table 2, show that the range of variation in the frontal shield index is from 1·25 to 1·6.

It is of interest to note that the colour of the three specimens of *P. porphyriacus* from the Herbert River, North Queensland, in the Macleay Museum (numbers 4, 12 and 20 in Table) were bleached, and that these snakes, which are generally so distinctive by reason of their colour, were only distinguishable from *P. australis* by the smaller number of their abdominal shields and the fact that they possessed fewer unpaired subcaudals.

The variation recorded in regard to this character within this well defined species suggests that it is unsafe to place reliance upon it for the differentiation of species, and establishes the fact that within a single species such variations as those recorded in the two series given in Table 1 are not unusual.

In view of this evidence we must conclude that no valid reason exists for the segregation of the Macleay specimens as a separate species.

Pseudechis cupreus.

The confusion which has arisen in regard to *P. cupreus* is particularly interesting. In 1865 Krefft described a specimen from the River Murray and a second specimen from Port Denison as *P. australis.* In 1887 McCoy described as *P. australis* and figured in colour a specimen from the Murray river which differed obviously from the typical *australis* in the possession of a single anterior temporal shield and of only 11 single anterior subcaudals.

Through the courtesy of Mr. Kershaw, Director of the Melbourne Museum, I have been able to examine the actual specimen from Kewell, Victoria, described and figured by McCoy. It coincides accurately in all respects with McCoy's description and figure, but has 10 maxillary teeth posterior to the poison fang (not two to five as in *Pseudechis*) and is unquestionably a *Demansia.* This explains the single anterior temporal shield.

Boulenger based his *cupreus* partly on McCoy's figured specimen and partly on a specimen from the Murray river incompletely described by Krefft, in 1865. Boulenger gives the length of *cupreus* as 1830 mm. (exactly that of McCoy's specimen). He gives 57 to 72 as the number of subcaudals, 11 to 34 being single. Both Krefft's specimens had 57 subcaudals, of which the anterior 34 were single. McCoy's specimen had a total of 72 subcaudals, of which only 11 were single.

Krefft later (1868) fully described his Murray river specimen and a second specimen from Port Denison, definitely identifying them with *P. australis*. Since Krefft's descriptions accurately fit *P. australis* there seems now no longer any reason to regard *P. cupreus* as a separate species.

CONCLUSIONS.

1. A series of *P. australis* from Cape York Peninsula and another of supposed *P. darwiniensis* in the Macleay Museum exhibit a graduation in the character used for differentiation of these species, i.e., the proportions of the frontal shield.

2. Similar variation in this character within a well-defined species of the same genus, *P. porphyriacus*, affords supporting evidence of its invalidity as a character for the differentiation of species.

3. *P. darwiniensis* is synonymous with *P. australis*.

4. Evidence is presented which destroys the claim of *P. cupreus* Boulenger to be regarded as a distinct species.

ACKNOWLEDGEMENTS.

My thanks are due to Professor W. J. Dakin, Professor of Zoology in the University of Sydney, for permission to examine the specimens in the Macleay Museum; and to Mr. Kershaw, Director of the National Museum, Melbourne, for the facilities he has given me for the examination of material; also to Mr. H. R. Rowan of the Lockhart River Mission, North Queensland, for much help in the field. Finally, I am much indebted to Mr. J. R. Kinghorn, of the Australian Museum, who examined some of my specimens, concurred with me in the identification as *P. australis*, and suggested that *P. cupreus* would probably be found to be identical with *P. australis*.

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OBSERVATIONS ON THE VENOM OF A LARGE AUSTRALIAN SNAKE, *PSEUDECHIS AUSTRALIS* (GRAY)

2. THE VENOM YIELDS AND VENOM.

by

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(From the Walter and Eliza Hall Institute, Melbourne).

(Submitted for publication 4th August, 1930.)

Pseudechis australis, next to *Demansia olivacea*, is probably the commonest large venomous snake on Cape York Peninsula. Here it is widely distributed, specimens having been taken on the Stewart and Lockhart Rivers on the east coast, at Coen in the interior and close to the mouth of the Coleman River, Gulf of Carpentaria. It chiefly frequents savannah, well grassed and swampy country, and takes refuge in holes in the ground. It is aggressive and when molested will fight savagely, rarely attempting to escape.

It is well known to the aborigines on the east coast as "Ngumai." Although they fear it as they do all snakes, venomous or otherwise, they do not regard it as being nearly as dangerous as another snake, "tai-pan," which shares its habitat. This is said to be more aggressive, more rapid in its movements and extremely deadly. It is said also to possess smaller scales and may possibly be identical with *Oxyuranus maclennani* Kinghorn.

(a) THE BITING APPARATUS OF *P. AUSTRALIS*.

Advantage was taken of the possession of a living specimen to apply the ingenious method of Fairley (2[a]) and procure moulds of the bite (Fig. 1). In the skull the distance between the fangs was 1·4 cm., but in the moulds of two snap bites the distances between the fang punctures were 2·0 and 2·15 cm. The distances between the palatine and pterygoid teeth were 0·8 cm. in the skull and 1·5 cm. at the bite. The fangs measured 6·5 mm. and the distance of penetration measured on a bristle inserted into the punctures, was 4 mm. In regard to length of fang, the snake is intermediate between Fairley's averages for the death adder *(Acanthopis antarcticus)* and the black snake *(Pseudechis porphyriacus)*. The fangs are much shorter than those of *Oxyuranus maclennani*. The association of wide separation of the fangs and large venom yields in this species agrees well

Figure 1. Impression of bite of *P. australis* (No. 6, Table 1).

with the generalization established by Fairley (2[a]) that in the Australian colubrines the average venom yields increase *pari passu* with increase in the interfang measurement. The great width of the head of this snake is due to the large venom glands and the powerful musculature surrounding them.

(b) THE VENOM YIELDS OF *P. AUSTRALIS*.

The two samples of venom obtained by Mr. McLennan from Specimens 2 and 3 in the first series of Table 1 of the preceding paper weighed about 0·2 gm. and 0·358 gm. respectively. They were procured by milking the snake immediately after it had been disabled.

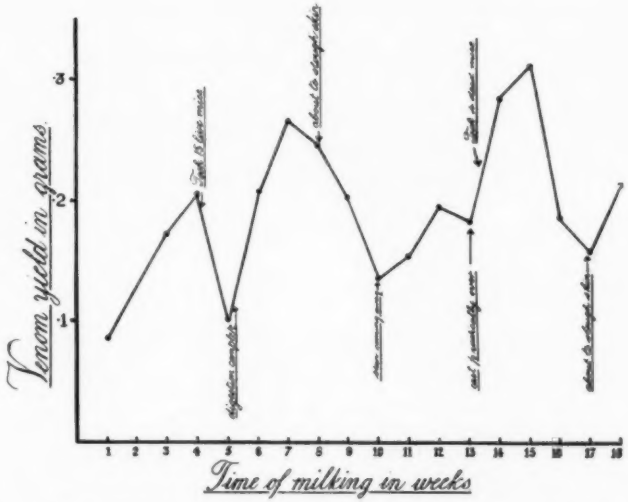

Figure 2. Graph of the venom yields of *P. australis* plotted against the time at which "milking" was carried out.

From Specimen 6 in the same table a series of weekly "milkings" were obtained in the field from August 14 to November 27, 1929. These were collected by allowing the snake to bite through a piece of rubber dam stretched over a glass and the venom was at once dried over calcium chloride in a field dessicator. The yields were finally re-dried *in vacuo* before weighing. The weights of venom yielded at these weekly milkings and at two of the subsequent milkings at Melbourne, together with the "primary" yield after capture on July 31, are set out in Table 1. The yields are shown graphically in Fig. 2. The primary yield 0·0859 gm. was low in comparison with the single yields obtained by McLennan. This is accounted for in part by the loss of venom during the capture of our

specimen and in part by the probability that McLennan's two specimens contain some saliva (as the snakes did not bite through rubber). The venom yield was small at the first weekly milking after the snake had fed for the first time in captivity but the next three milkings gave high values. The yields were low during the slough but rose again on its completion. A pronounced rise in the venom yields occurred after feeding when sloughing was complete. The whole series of observations form a striking demonstration of the rapidity with which venom may be secreted. Had the milkings been done at less frequent intervals it is possible that the individual yields might have been greater.

TABLE 1.

The Venom Yields of a Single Specimen of Pseudechis australis.

Date.	Weight of venom in grammes.	Observations.
31.7.29	0·0859	Much venom lost in capture of snake.
14.8.29	0·1719	This milking two weeks after capture was the first of the series of weekly milkings.
21.8.29	0·2051	Showed no interest in frogs and chickens placed in its cage but at once took mice which were offered to-day, consuming 15 in about two hours.
28.8.29	0·1004	On Sept. 2nd a 16th mouse was eaten. On Aug. 30th, eight days after feeding, faeces containing much mouse hair were observed in cage.
4.9.29	0·2079	
11.9.29	0·2659	
18.9.29	0·2459	Very sluggish, utmost difficulty in inducing it to bite, about to slough skin, eyes dull and opaque, orange coloured iris no longer visible.
25.9.29	0·2024	Still sluggish, skin not yet cast but is dry and loose.
2.10.29	0·1359	Snake sluggish, skin not yet cast.
9.10.29	0·1539	Snake more active, skin coming free over head.
16.10.29	0·1950	Very active, skin coming free over neck and body.
23.10.29	0·1816	Active, skin cast almost completed.
30.10.29	0·2844	Skin cast complete except for an odd patch on the neck. Four dead mice eaten during past week, though the snake would not touch live food. Live mice, as well as a small bandicoot, kept in the cage for days were refused.
6.11.29	0·3114	Largest single yield in series, followed upon skin slough and feed.
13.11.29	0·1844	
20.11.29	0·1560	Snake active but is about to slough skin again.
27.11.29	0·2138	Last of series of weekly milkings, extending over period of 16 weeks from 14.8.29 to 27.11.29.
30.12.29	0·2970	
6.1.30	0·0975	

(c) THE VENOM OF *P. AUSTRALIS*.

For the investigation of the venom we had available three samples; i and ii obtained by McLennan and Sample iii consisting of venom obtained from our living specimen. We relied upon this last for accurate information in regard to

the toxicity of the venom, using only the pooled "Melbourne" milkings, which were collected under nearly ideal conditions and were rapidly dried *in vacuo* over sulphuric acid at relatively low temperatures. The comparison of these three samples would, it was hoped, yield confirmatory evidence of the identification of the corresponding specimens. Sample i had already been examined by Kellaway and Williams (4) who had described it provisionally as the venom of *P. scutellatus* and had determined its approximate lethal dosage by intravenous injection in rabbits and by subcutaneous injection in guinea pigs and rats.

(i) THE TOXICITY OF THE VENOM.

The lethal action of the venom was studied in sheep, monkeys, cats, rabbits, guinea-pigs, rats and mice. For the observations on sheep the pooled venom obtained in Queensland from Snake 6 was used, but in the remaining experiments Sample iii consisting of Melbourne milkings from the same snake was used exclusively.

Observations on Sheep.—A few sheep were injected subcutaneously with venom in a concentration of 20 mgs. per cc. The injections were made in the fore leg close to the metacarpo-phalangeal articulation. The results are set out in Table 2.

TABLE 2.

Results of Subcutaneous Injection of the Venom of Pseudechis australis in Sheep.

Weight in kilos.	Dose in mgs. per kilo.	Result.
40	1·0	Died in 10 hours 20 minutes
27	0·8	Died in 3 hours
49	0·8	Died in 7 hours
33	0·6	Died in 11 hours
31·7	0·6	Died in between 13 and 19 hours
39	0·6	Died in 8½ hours
40·6	0·4	Died in between 13 and 19 hours

These results show that the venom is more toxic for sheep than that of *P. porphyriacus* for which Fairley's (2^b) experiments suggest a subcutaneous certainly lethal dose of 0·8 mg. per kilo. Fairley had one recovery among four animals injected with 0·6 mg. per kilo. of black snake venom and the death times observed with this dose were 27¾, 14, and 18¼ hours. A single animal received 0·8 mg. per kilo. and died in 24 hours, and another received 0·4 mg. per kilo. and died in 106 hours. The certainly lethal dose of the venom of *P. australis* is probably less than 0·6 mg. per kilo. Neurotoxic symptoms were conspicuously absent. The animals were at no time paralysed. The first obvious symptons were haemoglobinuria and respiratory distress and the animals became weak and collapsed at various intervals after the injection. The post-mortem appearances were very striking. There was blood-stained oedema round the site of injection and extending up the limb. The blood in the heart and great vessels was fluid and the intima

of the vessels was stained with blood pigment. The lungs were intensely congested with multiple haemorrhages of varying extent. There were haemorrhages variably placed in the liver, pancreas, adrenals and thyroid and in the mesentery. The small intestines were congested, and in several animals the spleen had a black tarry appearance on section. The kidneys were congested and stained with blood pigment. Intravascular haemolysis appeared to have played an important part in the fatal result.

Observations on Monkeys.—The few observations made suggest that for this species *(Macacus rhesus)* the venom is definitely less toxic than that of the common black snake. The results in four monkeys injected subcutaneously in the hind limb with the venom in a concentration of 20 mgs. per cc. are set out in Table 3.

TABLE 3.

Results of Subcutaneous Injection in Monkeys.

Weight in kilos.	Dose in mgs. per kilo.	Result.
3·04	1·5	Died in 13 hours 40 minutes
2·37	1·0	Very sick for two days but commenced to recover on the third day
3·35	0·8	Slight symptoms, recovery
2·61	0·5	No symptoms

The subcutaneous lethal dose for this species is probably rather more than 1·0 mg. per kilo. Black snake venom was lethal for monkeys in doses of 0·8 and 1·0 mg. per kilo. in between five and six hours, and even 0·5 mg. caused very severe symptoms (Kellaway, 3). With the venom of *P. australis* the symptoms observed in the animal which received 0·8 mg. per kilo. were so slight as to be hardly noticeable. The monkey went off its food for a few hours and seemed less fit than before the injection. A dose of 1·0 mg. per kilo. caused more severe symptoms. For two days the animal took no food but sat with his head bowed, taking but little interest in his surroundings. He was somewhat dyspnoeic, breathing with his mouth open. There was no definite weakness or paralysis though, during the latter hours of the first day, the eyelids tended to drop a little. There was no haemoglobinuria. The monkey, which received 1·5 mgs. per kilo., exhibited definite ptosis after five hours and was prostrate within eight hours. There was haemoglobinuria and some dyspnoea. In eleven hours it was profoundly collapsed and unconscious. Just before death there was bloodstained frothing at the nostrils and mouth, and the discharge of urine contained much blood pigment.

Post-mortem there was congestion and oedema at the site of the injection. The lungs were fleshy, sodden and haemorrhagic throughout. The blood in the heart and great vessels was intensely haemolyzed, very thin, and did not coagu-

late within 24 hours after being withdrawn. The spleen was almost black on section and the kidneys were intensely stained with haemoglobin. There was excess of bile-stained mucus in the small intestines.

Observations on Cats.—The cat is highly resistant to this as to other venoms. The results of subcutaneous injection in the tissues of the abdominal wall are set out in Table 4. The venom was injected in a concentration of 20 mgs. per cc.

TABLE 4.

Results of Subcutaneous Injection in Cats.

Weight in kilos.	Dose in mgs. per kilo.	Results.
1·54	7	Died in less than 46 hours
2·99	6	Died in less than 46 hours
2·65	5	Died on the fourth day
1·86	5	Died in 34½ hours
2·87	4	Moderate symptoms, survived
2·52	4	Died in less than 24 hours
2·65	3	Died in 78 hours
3·44	2	Slight symptoms, survived

The certainly lethal dose of this venom is probably about 5 mgs. per kilo., and for this species the venom appears to have about the same degree of toxicity as that of *P. porphyriacus*.

The symptoms were predominantly respiratory and at no stage were any definitely neurotoxic symptoms exhibited. The animals which succumbed appeared to be moderately well for the first 12 hours. They gradually became dyspnoeic and, though their movements were neither weak nor ataxic, they were disinclined for any exertion and when disturbed walked extremely slowly. Most of them had severe haemoglobinuria. Later they became collapsed and unconscious and their breathing was definitely dyspnoeic. They remained in this collapsed condition for several hours before death.

Post-mortem there was extensive haemorrhagic oedema spreading from the site of injection in the abdominal wall. The blood was partially haemolyzed and fluid, coagulating only after several hours *in vitro*. There were haemorrhages in the lungs, which were sodden and frequently somewhat collapsed. The kidneys were stained with altered blood and the urine contained much blood pigment and sometimes red blood cells also.

Observations on Rabbits.—In our first experiments with Sample iii we used two successive weighings each of 10 mgs. for some preliminary ranging experiments on wild rabbits. Although the separate milkings had been pooled we had omitted to powder the venom finely before mixing it, and these two weighings gave the strikingly different results set out in Table 5. The venom was injected intravenously in a concentration of 2 mgs. per cc.

TABLE 5.

Variation in Wild Rabbits in the Toxicity of Different Weighings of the Same Sample of Venom.

Number of weighing.	Weight of rabbits in kilos.	Dose in mgs. per kilo.	Total dose in mgs.	Result.
1	1·42	0·6	0·84	Died on the second day
	1·43	0·6	0·86	Died in 15 minutes
	1·40	0·5	0·70	Died in 2 hours 42 minutes
	1·51	0·5	0·76	Died in 1 hour 6 minutes
2	1·41	0·4	0·56	Died in 3 minutes
	1·41	0·4	0·56	Died in 3 minutes
	1·38	0·4	0·54	Died in 3 minutes
	1·42	0·2	0·28	Died in 1 hour 14 minutes

The variation in potency observed here appears to be outside the range of individual variation of resistance likely to be met with even in wild rabbits, which are not very satisfactory for determinations of toxicity as they tend to die without obvious cause and yield generally less uniform results than the domestic strains. As our venoms were very thoroughly dried and kept in a dessicator over calcium chloride up to the time of weighing, we can exclude variation in water content as the cause of this discrepancy. Such variation has not been observed with other more toxic venoms, but may reasonably be expected in those in which the active principles are presumably diluted to a large extent by inactive material. In all the other toxicity tests recorded here we have used this sample finely powdered and well mixed; under these conditions but little variation can be observed in separate weighings. The amount of individual variation normally met with is seen in a few observations upon wild rabbits into which a solution containing 2 mgs. per cc. of carefully pooled dry venom was injected intravenously. Four wild rabbits ranging in weight from 1·22 to 1·41 kilos. received 0·4 mg. per kilo. One died in 14 minutes, one in less than 16 hours, and two survived. Four wild rabbits ranging in weight from 1·13 to 1·57 kilos. received 0·5 mg. per kilo. They all died, one in 19 minutes, one in 1 hour 34 minutes, one in 3 hours 10 minutes, and one in 5 hours 35 minutes. The intravenous certainly lethal dose for wild rabbits is probably about 0·5 mg. per kilo. For domestic rabbits the results are set out in Table 6.

TABLE 6.

Results of Intravenous Injection in Domestic Rabbits.

Number of animals.	Average weight in kilos.	Dose in mgs. per kilo.	Result.
8	1·81	0·8	One died in 4 minutes, one in 33 minutes, one in 56 minutes, one in 1 hour, one in 1 hour 23 minutes, one in 2 hours, one in 2 hours 25 minutes, and one had severe symptoms but survived.
4	1·68	0·7	All survived after slight early symptoms.
2	1·72	0·6	Both survived.

The certainly lethal dose is not far removed from 0·8 mgs. per kilo. The symptoms and post-mortem findings were identical with those reported for Sample i (Kellaway and Williams, 4). The delay in coagulation time of the blood taken from the heart after death was very pronounced. One or two bloods did not coagulate in 20 hours, and in several clotting had not taken place within six hours, but had occurred by the following morning. The symptoms were weakness, incoordination of movements and slight dyspnoea, but paralysis of the limbs was not observed. There was nearly always a terminal convulsion. The rabbits surviving for longer than an hour exhibited intense haemoglobinuria. There was no evidence of any peripheral action on nerve endings, for when the phrenic nerves were stimulated with a faradic current immediately after death the diaphragm always responded briskly with the secondary coil at 30 to 35 cm. The dominant feature in the post-mortem changes was intense intravascular haemolysis with haemorrhages in the lungs and haemorrhagic congestion of the small intestine.

The fatal dose by subcutaneous injection is much greater. Three groups, each of four rabbits, received 1·5, 2, and 3 mgs. per kilo. subcutaneously in the abdominal wall in a concentration of 20 mgs. per cc. After 1·5 mgs. two died on the second day, after 2 mgs. one died 7 hours after the injection, and after 3 mgs. one died 6 hours after the injection, and one on the third day. The remainder of the animals survived after a period of sickness lasting one to two days. There were no neurotoxic symptoms, and only one or two of the animals which survived had haemoglobinuria. The intense local infiltration, haemorrhage, and haemolysis, spread from the site of injection through the subcutaneous tissues of the abdominal wall. The local damage appeared to ensure slow absorption and so protect the animals from the haemotoxic action of the venom. On the third or fourth day several rabbits opened up the local lesion by biting out an area of skin.

Observations on Guinea-pigs.—This venom injected subcutaneously into the abdominal wall in a concentration of 2·0 mgs. per cc. gave the results set out in Table 7.

TABLE 7.

Results of Subcutaneous Injection of the Venom of Pseudechis australis in Guinea-pigs.

Number of animals injected.	Average weight in gms.	Dose in mgs. per 100 gms.	Result.
19	254	0·16	One died in 4½ hours, three died in 5 hours, three died in 5½ hours, six died in 7 hours, two died in 7½ hours, three died in 9 hours, and one in 11½ hours.
10	265	0·12	Two died in less than 16 hours, two died in less than 19 hours, one in 28 hours, one in 29 hours, and four survived.
10	286	0·08	Four died in less than 16½ hours, one in 22 hours, one in 28 hours, and four survived.
10	285	0·04	All survived without symptoms.

The certainly lethal dose is about 0·16 mg. per 100 gms. The symptoms and post-mortem findings were identical with those already described by Kellaway and Williams (4) for Sample i, though this sample was definitely more potent. The delay in the coagulation time (one to two hours or more) of the blood taken from the heart immediately after death was very striking.

Observations on Rats.—The only rats available for this purpose were, unfortunately, larger animals than have been used with other venoms. A few observations were made by subcutaneous injection using the venom in a concentration of 1·0 mg. per cc. The injections were made in the flank. The results are set out in Table 8.

TABLE 8.

Results of Subcutaneous Injection in Rats.

Number of animals.	Average weight in gms.	Dose in mgs. per 100 gms.	Result.
12	188	0·3	One died in 7 hours and the remainder in less than 18 hours.
14	186	0·25	Twelve died on the first day, one on the second day, and one on the third day.
11	243	0·2	Seven died in less than 22 hours, three in 23½ hours, and one in 28 hours.
12	253	0·15	Two died in less than 22 hours, one in 27 hours, one in 27½ hours, four by the end of the second day, one on the fourth day, and three survived.

The certainly lethal dose for rats of this weight is about 0·2 mg. per 100 gms. or 2·0 mgs. per kilo. The symptoms and post-mortem findings were closely similar to those observed with Sample i, but this sample was definitely more active. The coat was ruffled, and the animals became weak and ataxic; paralysis was never observed. Haemoglobinuria was constant. The post-mortem findings were similar to those seen in other experimental animals. There was haemorrhagic oedema round the site of the injection. The blood was fluid and the coagulation time delayed (20 and 25 minutes). The lungs showed haemorrhages of varying degree. Occasionally there were haemorrhages in the adrenal glands. Congestion of the small intestine was invariable and frequently there was extensive haemorrhage into the lumen.

Observations on Mice.—The mice used were not all of one colour but were mixed white and coloured strains. For the larger doses the venom was used in a concentration of 0·1 mg. per cc., and for the smaller doses in a concentration of 0·05 mg. per cc. The results of intravenous injection are set out in Table 9.

TABLE 9.

The Results of Intravenous Injection in Mice.

Number of animals.	Average weight in gms.	Dose in mgs. per 20 gms.	Result.
2	22	0·06	Died in 16 and 23 minutes.
2	22	0·04	Both died in 23 minutes.
2	24	0·02	Died in 33 and 39 minutes.
4	23	0·01	All died in less than 18 hours.
12	23	0·008	Six died in 25, 30, 35, 39, 48, and 50 minutes; one died in 1¼ hours, two in 1¾ hours, one in 3¾ hours, and one in 17 hours and one on the second day.
10	21	0·006	Three died on the first day, one on the second day, and six survived.
10	23	0·004	One died on the first day, three on the second day, and six survived.
4	25	0·002	All survived without symptoms.

The certainly lethal dose appears to be about 0·008 mg. per 20 gms. or 0·4 mg. per kilo. With the larger doses the symptoms were strikingly uniform, and these and the post-mortem findings were similar to those observed in other species of experimental animals. There was no immediate shock suggestive of thrombosis, but symptoms came on within a few minutes. The animals were at first weak and ataxic and later collapsed. There was dyspnoea, and in one or two animals haemorrhage from the lungs. With the smaller doses and more prolonged death-times haemoglobinuria was invariable.

On post-mortem examination the blood was fluid in the heart and vessels. The lungs showed haemorrhages of varying degree. The small bowel was congested. The kidneys were congested and stained by haemolysis and blood pigment was present in the urine.

The effects of subcutaneous injection in mice are set out in Table 10. The venom was administered in a concentration of 0·1 mg. per cc.

TABLE 10.

The Results of Subcutaneous Injection in Mice.

Number of animals.	Average weight in gms.	Dose in mgs. per 20 gms.	Result.
21	19	0·06	One died in 4¼ hours, one in 4¾ hours, one in 6 hours, two in 7½ hours, two in less than 18 hours, thirteen in less than 20 hours, and one in 24 hours.
21	22	0·04	Sixteen died on the first day, one on the second day, and four survived.
15	23	0·03	Four died on the first day, one on the second day, one on the third day, one on the fourth day, and eight survived.
10	19	0·02	Two died on the first day, two on the second day, and six survived.

The certainly lethal dose by subcutaneous injection is about 0·06 mgm. per 20 gms. or 3 mgs. per kilo. The subcutaneous intravenous index of the venom for mice is about 7·0. The symptoms closely resemble those in other experimental animals. No paralysis was observed, but there were dyspnoea, weakness and ataxia, and finally collapse. Haemoglobinuria was almost invariable.

On post-mortem examination the abdominal wall at the site of the injection was infiltrated with bloodstained oedema. There were haemorrhages in the lungs and the small bowel was intensely congested. The peritoneum was wet and stained with blood pigment. The blood was fluid in those animals observed shortly after death.

(ii) COMPARISON OF THE THREE SAMPLES OF VENOM.

We have compared the action of the three samples of venom by coagulation tests *in vitro*, by haemolysis tests, by studies of changes in blood pressure following intravenous injection in intact anaesthetized rabbits and by their action on the isolated rabbit auricle.

(a) *Coagulation Tests in vitro.*

All three samples cause a striking lengthening of the coagulation time of the blood of rabbits killed by intravenous injection and none, even in large doses, gives rise to intravascular coagulation. The venom in doses ranging from 2 mgs. to $1·0 \times 10^{-9}$ mgs. fails to cause any coagulation *in vitro* when added in a volume of 0·1 cc. of saline to 0·2 cc. of citrated plasma. This was tested for us by Miss Williams using human citrated plasma and citrated plasmas of the sheep, rabbit, and guinea-pig. The plasmas were prepared by shedding blood into an equal volume of 2% solution of sodium citrate in physiological saline.

Further tests were made to ascertain the anticoagulant power of the samples of venom, using the technique of Lamb (5) and adding that amount of 0·5 or 1% solution of calcium chloride which causes rapid clotting in control tubes containing 0·1 cc. of saline and 0·2 cc. of plasma. The results of these tests in all the plasmas were exceedingly uniform. The tubes containing venom and plasma, and the control tubes containing plasma and saline were incubated for 1 hour at 37°C. before adding calcium chloride. They were then observed at room temperature, 18° to 20°C. The results with rabbit plasma are set out in Table 11.

TABLE 11.

The Anticoagulant Power of the Three Samples of Venom. Clotting Times after the Addition of Ca Cl_2.

Dose of venom in mgs.	Clotting times after the addition of $CaCl_2$.							
	0·1	0·01	0·001	0·0001	0·00001	0·000001	0·0000001	Control
Sample i	N	N	N	N	30 mins., soft	15 mins.	9½ mins.	9½ mins.
Sample ii	N	N	30 mins.	16 mins.	12 mins.	9 mins.	8 mins.	8 mins.
Sample iii	N	N	N	2 hrs.	26 mins.	16 mins.	15 mins.	11 mins.

N=no coagulation in 20 hours.

The results with guinea-pig, human and sheep plasmas were closely similar to those observed with rabbit plasma. In all the tests Sample iii was a little less potent than Sample i and Sample ii was very definitely inferior to both i and iii in anti-coagulant power.

(b) *Haemolysis Tests.*

These were made, using thoroughly washed red blood cells of the guinea-pig, rabbit, and man. The total volume of the haemolytic system was 0·3 cc. (0·1 cc. of saline, 0·1 cc. of saline containing venom and 0·1 cc. of 3% suspension of red blood corpuscles). The venom was used in halving dilutions—0·1, 0·05 . . . . 0·000195 mgs. (expressed in Table 12 as the nearest significant figure). In the table the earliest time at which complete haemolysis was observed in each tube is set out. The tubes were observed for three hours at 37°C. and thereafter at room temperature, 16 to 20°C. No haemolysis was observed in any of the controls.

TABLE 12.

Times at which Complete Haemolysis was observed, using Halving Dilutions of the Three Samples of Venom and Washed Human, Rabbit, and Guinea-pig Red Blood Corpuscles.

	Time in hours.									
Human Red Cells:	0·1	0·05	0·025	0·0125	0·006	0·003	0·0016	0·0008	0·0004	0·0002
Sample i	¼	½	1¼	1¾	2	2½	3	20	20	20
Sample ii	I	N	N	N	N	N	N	N	N	N
Sample iii	½	1	2¼	2½	2½	3	3½	N	N	—
Rabbit Red Cells:										
Sample i	¾	1½	2	2	2½	2½	2¾	N	N	N
Sample ii	I	I	I	I	N	N	N	—	—	—
Sample iii	1½	2	3½	4	4	4	4	N	N	—
Guinea-pig Red Cells:										
*Sample i	½	¾	1	1½	2	—	—	—	—	—
Sample ii	3	3	20	20	20	20	N	N	—	—
Sample iii	½	¾	¾	1	1½	1½	2½	2½	2½	—

*Put up also in 1/10 dilutions and haemolyzed to 0·0001 in 3 hours.

N=no haemolysis. I=incomplete haemolysis. — = not tested.

Sample ii is greatly inferior to the other two samples of venom in haemolytic power. This is rather surprising since venom haemolysin is extremely stable and is not readily affected by heat except in extreme dilution. It is, however, rather sensitive to acid. Mr. Holden kindly determined electrometrically the pH of solutions containing 5 mg. per cc. That of Sample i was 6·4, of Sample ii 5·6, and of Sample iii 6·2. As Sample ii was kept in the field for a whole wet season, and as it was not perfectly dry when it reached us more than a year after it was collected, it seems not unlikely that even the low degree of acidity observed (doubtless produced by bacterial action) may be sufficient to account for the deterioration of the sample.

Cultures of this sample grew a non-haemolytic streptococcus with which a solution of Sample iii (5 mgs. per cc.) was inoculated. After standing a fortnight at room temperature, 16° to 20°C., the pH of the contaminated solution was approximately 5·6. The venom now caused complete haemolysis of 3% washed guinea-pig corpuscles in amounts of 0·1 . . . . 0·00039 mgs. in 3 hours, but there was striking delay in haemolysis, none occurring in any of the tubes during the first 1½ hours. 1·0 mg. of this altered venom failed to cause haemolysis in 3 hours.

(c) *Changes in the Blood Pressure of Rabbits.*

These were investigated for all three samples in domestic rabbits under ether anaesthesia with the vagi divided or intact and with or without artificial respiration. Samples i and iii exhibited identical behaviour and the changes observed were exactly those described for Sample i by Kellaway and Williams (4). The venom injected intravenously in doses ranging from 4 to 8 mgs. invariably causes a rapid but transitory fall of blood pressure. During the succeeding 15 to 60 minutes there is a period of raised blood pressure during the latter part of which there are occasional convulsions which appear to originate Traube curves. The blood in the carotid cannula remains bright in colour until the end of the experiment, when respiration fails and the blood pressure rapidly falls. The rise in blood pressure and the Traube curves are possibly asphyxial in origin, though they may be due to direct action of the venom on the bulbar centres. If the phenomena are asphyxial it is probable that they are determined by some alteration in the exchange of oxygen between the red blood corpuscles and the tissues, since the extent of the haemolysis of red blood cells is hardly sufficient to cause them. Some part in the terminal fall is undoubtedly played by the direct action of the venom on the heart muscle which will shortly be described. The phenomena require further analysis, but for the present they are being used only for the comparison of samples of the venom, and point clearly to the identity of Samples i and iii.

The post-mortem changes observed in the rabbits are very striking. The blood is practically incoagulable, samples drawn from the great veins after death failing to clot in 24 hours. The urine in the bladder contains blood pigment. The parietal peritoneum is stained by haemolysis, the intestines are congested, and the lungs are usually haemorrhagic. In one or two experiments the animals died of acute pulmonary oedema.

The changes following the injection of Sample ii were strikingly different. The venom was injected intravenously in doses ranging from 5 to 30 mgs. The blood pressure fell after the injection and never again recovered. At no time was there any period of high pressure with Traube waves. The blood pressure of animals respiring naturally showed striking increase in the respiratory excursion, but the blood pressure progressively fell until the respirations finally failed or

the animal died of pulmonary oedema. In these rabbits there was no striking haemolysis. At autopsy the urine contained blood, and there were haemorrhages into the lumen of the bowel, in the heart muscle (sometimes with haemopericardium), in the lungs, adrenals, mesentery, and in the skeletal musculature. A similar picture was presented in unanaesthetized rabbits dying after the intravenous injection of the venom. The certainly lethal dose was much higher than that of Samples i and iii, both in rabbits and rats. In rabbits doses of 2 and 3 mgs. per kilo. injected intravenously failed to kill, and those of 4 and 5 mgs. killed in 2 hours and in 1 hour and 40 minutes. In rats 3 mgs. per 100 gms. injected subcutaneously killed on the second day, but animals injected with doses of 2·5 and 2·0 mgs. per 100 gms. survived. In the rats which died the post-mortem findings were similar to those observed with this sample of venom in rabbits. In all these animals there was prolonged delay in the clotting time of blood taken immediately after death.

In view of the bacterial contamination of the venom and its subjection to a prolonged period of heat and moisture we cannot regard these observed facts as evidence that the sample of venom comes from a species other than *P. australis*. The totally different post-mortem picture is probably accounted for by the fact that the animals instead of being killed acutely by the action of the venom on the blood corpuscles, on the heart muscle, and on the bulbar centres, are killed more slowly by the action of a "haemorrhagin" present in the unaltered venom, but only exhibiting a dominant action when the venom is injected in large amounts and allowed sufficient time to act owing to the loss of its haemolytic power.

The streptococcus isolated from Sample ii when grown in a solution of Sample iii for a fortnight at laboratory temperatures did not suffice to parallel this result. Injected intravenously in a dose of 5 mgs. into a rabbit under ether anaesthesia this slightly altered venom caused the blood pressure changes and post-mortem effects characteristic of the unaltered venom.

(d) *The Action of the Venom on the Isolated Auricle of the Wild Rabbit.*

Whereas cobra venom has a powerful and rapid action on the isolated rabbit's auricle most of the Australian venoms so far tested have but little effect. An exception is provided by the venom of *P. australis*, which causes cessation of the beat in a concentration of 1 in 10,000 in about an hour. In Table 13 are summarized the effects of various concentrations of the three samples on the isolated auricle of the wild rabbit suspended in a bath containing 100 cc. of oxygenated Trevan's solution as modified by Burn (1). We used the isolated organ bath devised by Dale and Laidlaw and carried out the experiments at 35°C. In Trevan's borax-boric acid Ringer's solution the rabbit auricle normally beats for some hours if *vigorous* oxygenation be provided.

TABLE 13.

Effect of the Venoms on the Isolated Auricle.

Sample.	Concentration.	Result.
i	1 in 5,000	Cessation of beat in 1 hour.
i	1 in 5,000	Cessation of beat in 2 hours and 14 minutes.
i	1 in 10,000	Beating very feebly after 47 minutes.
ii	1 in 7,500	Auricle still vigorous after 1 hour.
iii	1 in 5,000	Cessation of beat in 27 minutes.
iii	1 in 5,000	Cessation of beat in 55 minutes.
iii	1 in 10,000	Cessation of beat in 1 hour 6 minutes.

Some experiments were also made with the isolated wild rabbit heart perfused with the same oxygenated Ringer's solution. Since no striking alteration in the rate of flow through the coronary circulation was observed with Sample iii in a concentration of 1 in 30,000, though the heart had practically ceased to beat after a little more than an hour, we did not use this method for the comparison of the samples, but relied on the tests with the isolated auricle. These provide further evidence of the identity of Samples i and iii.

(e) *Summary of the Evidence in Regard to the Identity of the Samples.*

The evidence of the identity of Samples i and iii is very complete. They produce identical symptoms and post-mortem changes when injected subcutaneously in guinea-pigs and rats, and intravenously in rabbits. They are almost equally powerful in haemolytic action, both *in vitro* and *in vivo*, and in both they exhibit an anticoagulant action. They cause identical and peculiar changes in the blood pressure of the anaesthetized rabbit, and have a similar action in causing cessation of the beat of the isolated rabbit auricle.

The somewhat higher potency of Sample iii is accounted for by its collection uncontaminated by saliva and by its rapid drying under conditions unfavourable to bacterial action. It should, however, be remarked that samples of venom from individual snakes of the same species or from the same snake at different times are not necessarily equally potent, particularly if the venom concerned be of relatively low potency. Some evidence in this regard has been offered in the present communication.

Sample ii resembles the other two in its haemolytic and anticoagulant power, though its haemolytic power is much feebler than that of i and iii. It differs from them in its haemorrhagic action in the large doses necessary to kill experimental animals, and in its effects on the blood pressure in anaesthetized rabbits. In view of the ascertained facts concerning its collection and history the examination of this sample cannot be regarded as affording evidence either for or against the view that the specimen from which it was collected is of the same species as those yielding i and iii. The opportunity afforded for bacterial action throughout a wet season in the tropics and its excessive acidity suggest that it is indeed the same venom as i and iii, but has been altered, notably by the destruction of its haemolytic power.

(iii) THE TOXIC CONSTITUTION OF THE VENOM OF *P. AUSTRALIS*.

The observations recorded in this paper, though by no means complete, afford a good deal of information in regard to the toxicity and toxic constitution of this venom. In Table 14 the certainly lethal doses of the venom for various species are summarized.

TABLE 14.

Certainly Lethal Dosage of the Venom of P. australis for Laboratory Animals.

Species.	Lethal dose in mgs. per kilo. Subcutaneous.	Intravenous.
Sheep	less than 0·6	—
Monkey	more than 1·0	—
Cat	about 5·0	—
Rabbit		more than 0·8
Guinea-pig	1·6	—
Rat	2·0	—
Mouse	3·0	0·4

The venom is much less toxic than those of *Notechis scutatus* (the tiger snake), *Acanthopis antarcticus* (the death adder), *Denisonia superba* (the copper head), *Oxyuranus maclennani* and *Demansia textilis* (the brown snake). It is of the same order of toxicity as those of two other species in the same genus, *Pseudechis porphyriacus* (the common black snake) and *Pseudechis guttatus* (Kellaway, 3). It also resembles these last venoms in its powerful haemolytic action, but differs from them in the absence of a thrombase, possessing, on the contrary, a more powerful anticoagulant action than the venom of any other Australian snake so far investigated. The presence or absence of a thrombase cannot therefore be regarded as a generic character.

There seems to be little evidence of any neurotoxic action by this venom, the only hints of such action being afforded by the ptosis which occurs as an early symptom in monkeys, the effects on the blood pressure of rabbits, and the failure of respiration which in some species is the terminal event following its injection. Unlike most other Australian venoms it has a striking action on the heart muscle. Possibly the venom contains a haemorrhagin though this, if present, plays no striking rôle in causing death.

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